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A Concept of a Probable Autoinjector for Bio-threat Agents

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ABSTRACT

Chemical and biological weapons can be used during conflicts and by terrorists to injure or kill humans and animals. Chemical weapons contain toxic chemicals and biological weapons contain pathogenic organisms. With proper protective equipments and training, the lethal effects of chemical and biological warfare agents can be minimised. First aid kit is available that contain detection, decontamination and medical protection for chemical warfare agents including autoinjectors, for rapid administration and faster absorption of drugs. The autoinjectors are safe and available for life saving drugs like atropine sulphate and pralidoxime chloride (nerve agent poisoning), epinephrine (anaphylaxis), diazepam (seizures) and sumatriptan (migraine). For bio-threat agents doxycycline alone is available as a broad spectrum antibiotic in the first aid kit. Majority of the bacterial agents are gram negative and hence amikacin drug cartridge was developed for the existing autoinjector. The advantage of amikacin is its safety, stability, can be given prophylactically and supplemented with other antibiotics when medical attention arrives. The usability and tolerability of amikacin administered repeatedly through autoinjector was studied using various haematological, biochemical and oxidative stress parameters in animal models. The results are promising and as there is no antibiotic autoinjector available, amikacin autoinjector can be considered for bio-threat agents.

Keywords : Autoinjector, bio-threat agents, amikacin, oxidative stress

1. INTRODUCTION

The use of chemical and biological warfare agents on humans, animals and plants to cause harm or death is a concept followed by several State Parties and by terrorist organisations[1]. The chemical weapons can cause death and injury through its chemical action on the biological system[2]. The biological weapons are bacterial and viral agents that are pathogenic to humans and animals, and toxins derived by biological sources[3]. The chemical agents act very fast and its detection and protection has to be carried out very quickly. Defence Research and Development Organisation has developed a first aid kit that contains detection, decontamination and medical protection[4]. The nerve agents like tabun, sarin, soman and VX act rapidly and hence for medical protection the antidotes are provided as autoinjectors in the first aid kit, for rapid administration and faster absorption of drugs.

The autoinjectors are very safe. When given intramuscularly the autoinjector can deliver the drugs faster with rapid absorption compared to manual injection due to the spray effect of drug delivery in autoinjector. In the autoinjector, the needle is not visible outside and hence do not cause any fear. The injection will be painless, easy to administer and well suited for mass casualty management. When healthy volunteers were administered atropine and pralidoxime chloride through autoinjectors, the drug absorption was faster by the autoinjector[5]. Other than atropine sulphate and pralidoxime chloride (nerve agent antidotes), autoinjectors are available for few life saving situations viz., epinephrine for anaphylaxis, diazepam for status epilepticus, sumatriptan for migraine and midazolam for convulsions[6].

For bio-threat agents in the first aid kit, doxycycline alone is available as a broad spectrum antibiotic for oral administration. During training and operation in the military service, low intensity conflicts, road accidents and also in natural disaster situations, serious injury may occur for which an antibiotic may be required. An autoinjector will be very useful in such situations. In addition, the antibacterial autoinjector can also be used as an antidote for bio-threat agents. A concept of development of a probable autoinjector for an antibacterial drug and an analgesic drug was earlier proposed[6,7]. Previously, amikacin drug cartridge was developed for the existing reusable autoinjector and tested in animal models for its tolerance and usability using various haematological and biochemical parameters[8]. All parameters viz., liver function test, kidney function test, serum enzymes and general biochemical variables did not show any significant change[9]. Since the autoinjector delivers the drug with force, in the present study amikacin was delivered intraperitoneally in rats by autoinjector and compared with manual injection for various oxidative stress parameters. Since, amikacin will be effective against most of the gram negative organisms and few gram positive organisms, an *in vitro* study was also carried out using combination of antibiotics for its effectiveness for future development[10].

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2. MATERIAL AND METHODS

2.1. Animals

Female Wistar rats weighing 120 g - 180 g from Biomedical Research Unit and Laboratory Animal Centre, Saveetha University were used. The animals were housed in polypropylene cages, three per cage with dust free paddy husk as the bedding material. They were provided with laboratory animal feed (VRK Nutritional, India) and filtered water *ad libitum*. The animals were maintained at a temperature of 25 ± 2 °C and natural dark/light cycle. The study was approved by Institutional Animal Ethical Committee of Saveetha University (SU/ BRULAC/RD/010/2014).

2.2. Drug Cartridge and Dilution

Amikacin drug cartridge developed by Saveetha University with the collaboration of Defence Research and Development Organisation and M/s Neon Laboratories (Mumbai), was used for the study (as shown in Fig. 1). The drug cartridge was developed with human dosage of 250 mg/mL (2.35 mL/vial). For the animal administration, the human dose cartridge was diluted 1:4, to make the concentration to 63 mg/mL. For this, 1.75 mL of the drug solution was withdrawn from the cartridge in sterile condition using a laminar flow and 1.75 mL of sterile saline was injected back. The autoinjector capable of delivering full dose with full needle out or partial dose with partial needle out was used[9]. The drug cartridge was loaded in the autoinjector with the plastic clip restrictor for partial dose delivery (1.2 mL against 2.0 mL) and partial needle out (15 mm against 25 mm), since it was an animal study through intraperitoneal route[9]. The refilled amikacin cartridges were subjected to a variety of quality control parameters viz., sterility test, firing efficacy test, stability test, low pressure test and vibration test.

2.3. Experimental Groups

Thirty five female rats were randomly allocated to the following five groups (7 rats per group).

Group 1 : Control
Group 2 : Amikacin, 63 mg/mL (1.2 mL, i.p.) for 3 days by autoinjector,
Group 3 : Amikacin, 63 mg/mL (1.2 mL, i.p.) for 3 days by manual injection.
Group 4 : Amikacin, 63 mg/mL (1.2 mL, i.p.) for 7 days by autoinjector
Group 5 : Amikacin, 63 mg/mL (1.2 mL, i.p.) for 7 days by manual injection.

The rats were held on the surgical table with its back on the table gently and firmly. The autoinjector was unlocked and positioned gently on the lower abdomen vertically. The trigger button was pressed and held on to the abdomen for 10 seconds. The drug cartridge was weighed before and after injection to estimate the quantity of the drug delivered. For the manual injection, the drug solution that was removed from the cartridge, was diluted suitably with sterile normal saline to get a concentration of 63 mg/mL and from that 1.2 mL was injected intraperitoneally (i.p.) to each rat.

2.4. Sample Collection

The animals were weighed daily. General behaviour, food and water intake were recorded. Twenty four hours after the last dose (4th or 8th day), the animals were anaesthetised with isoflurane and blood was collected from the orbital sinus in two separate tubes, one with anticoagulant (K_3EDTA) and the other without anticoagulant. After the collection of the blood, the animals were sacrificed with a over dose of anaesthesia. Vital organs like heart, lungs, spleen, kidney and liver were excised, blotted free of blood, weighed and preserved in formalin solution.

2.5. Oxidative Stress Parameters in Blood

Catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD) and glutathione reductase (GR), were estimated using the haemolysate. From the tubes without anticoagulant, serum was separated and was used for the estimation of malondialdehyde (MDA) and reduced glutathione (GSH). Roche's diagnostic kits (Chennai, India) were used and the assays were carried out as per the manufacturer's instruction (expressed as units), in a spectrophotometer (Shimadzu, Japan).

2.6. *In vitro* Test for Combination of Antibacterial Agents

Disc diffusion test was performed according to the NCCLS procedure[11]. The lawn culture of the test organisms were compared to 0.5 Mac Farland standard made on Mueller Hinton agar (HiMedia, India)[12]. The antibacterial activity of the antibiotics were tested alone and also in combination. The plates were incubated at 37 °C for 24 h and the zone of inhibition was measured. Two strains were used for the *in vitro* sensitivity test, (i) *Pseudomonas aeruginosa* as gram negative and (ii) *Staphylococcus aureus* as gram positive. Amikacin alone and in combination with cefazolin and vancomycin were tested. The tests were done in triplicates.

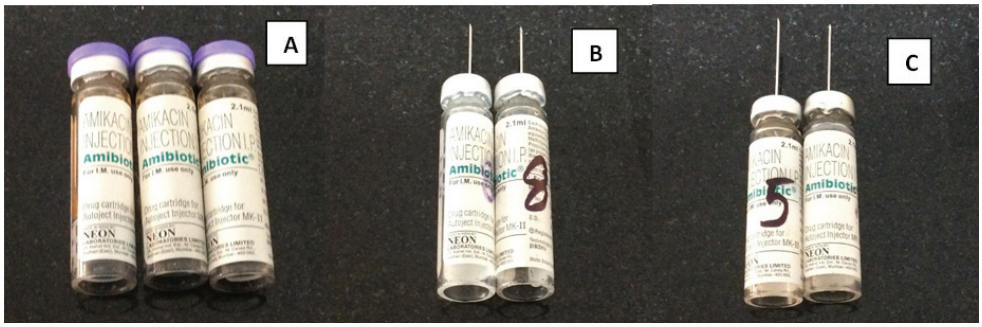

Figure 1. Amikacin drug cartridges for reusable autoinjector, (a) fresh cartridges, (b) partially ejected drug cartridges, and (c) fully ejected drug cartridges.

2.7. Statistical Analysis

All the parameters were analysed using one way analysis of variance (ANOVA) 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 (SysStat Software Inc., USA).

3. RESULTS

All the animals survived in all the groups. Bleeding or abscess was not observed in any of the groups. The food and water intake were also apparently similar in all the groups. The approximate dose of amikacin administered was 500 mg/kg and the effective needle length was 15.5 mm. The refilled cartridges complied with all the quality control parameters.

There was no significant change in blood GSH and MDA levels in 3 d and 7 d groups of autoinjector and manual injection when compared to control group as shown in Fig. 2. Similarly, no significant change was observed in blood SOD, CAT, GPX and GR levels in 3 d and 7 d groups of autoinjector and manual injection when compared to control group as shown in Figs. 3 and 4.

The results of *in vitro* test for the antibiotics and their combination against gram negative and gram positive organisms are given in Table 1. In the case of gram negative organism, amikacin alone or its combination with cefazolin or vancomycin showed no statistically significant difference, suggesting that amikacin alone is adequate. The combination did not show any antagonist effect against the gram negative organism. But, in the case of gram positive organism, combination with cefazolin or vancomycin showed statistically significant effect.

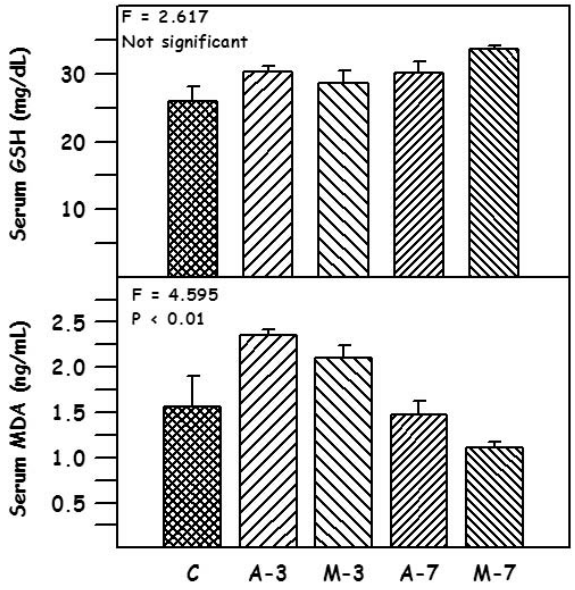

Figure 2. Effect of amikacin administered through autoinjector and manual injection by i.p. in rat on reduced glutathione (GSH) and malondialdehyde (MDA), C = Control Group; A-3 = autoinjector 3 doses; M-3 = manual injection 3 doses; A-7=autoinjector 7 doses; M-7 = manual injection 7 doses; Mean + SE (n = 7) one way ANOVA followed by Dunnett's comparison test with control group was carried out.

4. DISCUSSION

Several field equipments are available for the rapid detection of chemical agents and protection can be taken immediately. Additionally, medical protection can be taken using the first aid kit[4]. For the detection of the bio-threat

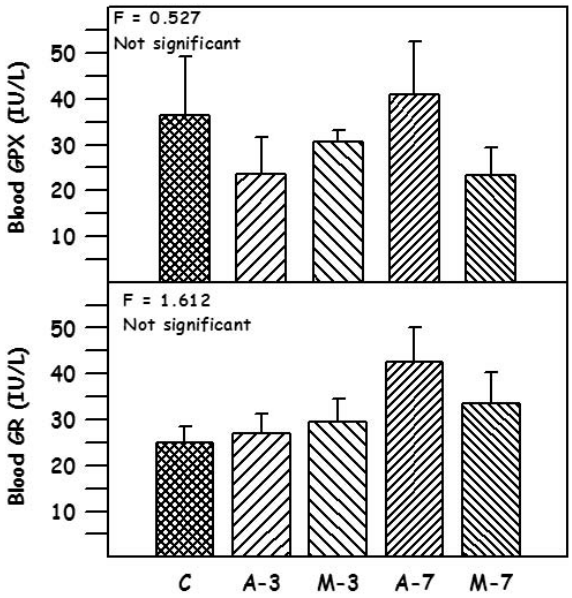

Figure 3. Effect of amikacin administered through autoinjector and manual injection by i.p. in rat on superoxide dismutase (SOD) and catalase (CAT), C = Control Group; A-3 = autoinjector 3 doses; M-3 = manual injection 3 doses; A-7 = autoinjector 7 doses; M-7 = manual injection 7 doses; Mean + SE (n = 7), one way ANOVA followed by Dunnett's comparison test with control group was carried out.

Table 1 . *In vitro* antibacterial activity of antibiotics individually and in combination against *Pseudomonas aeruginosa and Staphylococcus aureus*

Organism	Antibiotic	Zone of inhibition (mm) Mean ± SE (n=3)
Pseudomonas aueroginosa (Gram negative)	Amikacin	22.14 ± 0.48
	Cefazolin	19.55 ± 0.78
	Vancomycin	20.76 ± 1.05
	Amikacin + Cefazolin	22.25 ± 1.21
	Amikacin +Vancomycin	23.35 ± 0.69
F = 2.80 P = 0.085		
Staphylococcus aureus (Gram positive)	Amikacin	18.25 ± 0.72
	Cefazolin	21.67 ± 0.69*
	Vancomycin	25.42 ± 0.46*
	Amikacin+Cefazolin	22.85 ± 0.90*
	Amikacin +Vancomycin	26.25 ± 0.95*
F = 17.44 P < 0.001 *Statistically significant from amikacin		

Table 2 . Characteristics of bacterial bio threat organisms in categories A, B and C, and their drug of choice

Organism	Lethality	Human exposure	Grams reaction	Drugs -First choice	Drugs- other alternatives
Category A – Bacterial agents					
Bacillus anthrax	High	Aerosol	Gram positive	Ciprofloxacin, Doxycycline	Clindamycin, Rifampicin, Penicillin G
Yersnia pestis	High	Aerosol	Gram negative	Streptomycin, Doxycycline, Amikacin[17]	Ciprofloxacin, Co trimaxazole, Gentamycin, Chloramphenicol
Francisella tularensis	Moderate	Aerosol	Gram negative	Gentamycin, Chloramphenicol, Amikacin[18]	Ciprofloxacin, Doxycycline, Levofloxacin
Category B – Bacterial agents					
Coxiella burnetti	Low	Aerosol	Gram negative	Doxycycline	Tetracycline
Brucella species	Moderate		Gram negative	Doxycycline+ Streptomycin/ Gentamycin/ Amikacin[19]	Doxycycline+ Rifampicin Cotrimoxazole+ Rifampicin Ciprofloxacin+ Rifampicin
Burkholderia mallei	Low	Aerosol	Gram negative	Cephalosporins Co trimoxazole Tetracycline	Aminoglycosides Amikacin[20]
Rickettsia prowazekii	Low		Gram negative	Doxycycline Tetracycline	Chloramphenicol Erythromycin
Salmonella Species	High to moderate	Food and water	Gram negative	Fluoroquinolones Chloramphenicol	Cefoperazone, Ceftriaxone Cotrimoxazole Amoxicillin Azithromycin
Shigella dysentriae	Low	Food and water	Gram negative	Ciprofloxacin	Azithromycin, Ceftriaxone, Aminoglycosides Amikacin[21]
Escherichia coli	Low	Food and water	Gram negative	Fluoroquinolones	Amoxycillin+ Clavulanic acid Amikacin[15] Cephalosporin, Tigecycline, Polymyxin B
Vibrio cholerae	High if untreated	Food and water	Gram negative	Doxycycline, Tetracycline	Cotrimoxazole, Chloramphenicol Fluoroquinolone Azithromycin, Amikacin[22]
Category C – Bacterial agents					
Mycobacterium tuberculosis MDR strain	High	Aerosol	Acid fast bacterium	Rifampicin Isoniazid Streptomycin	Quinolones Amikacin[23]

agents, field equipments are available but, their specificity is always questionable[13]. In such a situation, a prophylactic drug is essential and amikacin can be administered even in doubt. Table 2 lists the bio-threat agents as per Centers for Disease Control and Prevention (CDC) classification[14]. Category A includes bacterial agents that possess high risk to the public and national security. They can be easily spread or transmitted from person to person and result in high death rates and can cause public panic. Category B includes agents that are of second priority. They are easy to spread and produce moderate illness and low lethality. Whereas, category C includes agents which are emerging pathogens that could be engineered for mass spread in the future. They may cause high morbidity and mortality rates and major health impact. Table 3 shows the bacterial agents, which are associated with field infections viz., military operations, terrorism, accidents and natural disasters[14].

The drug of choice for each agent is also given in Tables 2 and 3. For the majority of the agents, particularly gram negative organisms, if a single antibacterial drug is required the preference is for amikacin. Hence, amikacin drug cartridge was developed for the existing autoinjector. This can be supplemented with oral doxycycline available in the first aid kit. In the present study, amikacin was tested against common field bacteria (gram negative and gram positive), *in vitro* for its effectiveness. Amikacin is an amino glycoside antibiotic derived

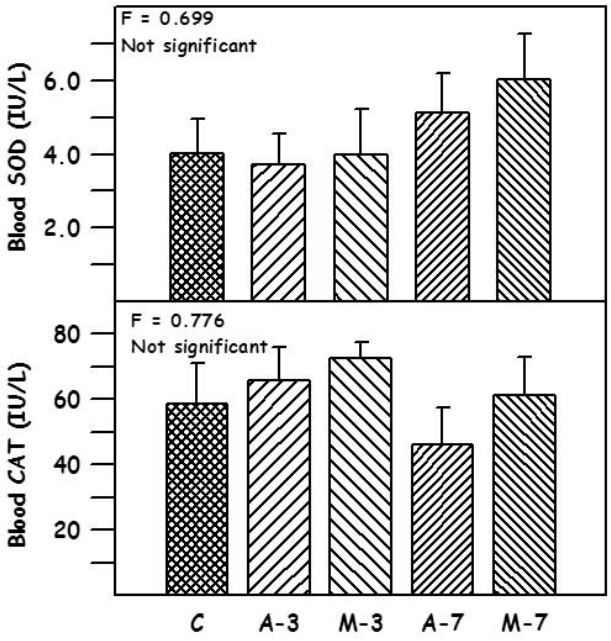

Figure 4. Effect of amikacin administered through autoinjector and manual injection by i.p. in rat on glutathione peroxidase (GPX) and glutathione reductase (GR), C = Control Group; A-3 = autoinjector 3 doses; M-3 = manual injection 3 doses; A-7 = autoinjector 7 doses; M-7 = manual injection 7 doses; Mean + SE (n = 7), one way ANOVA followed by Dunnett's comparison test with control group was carried out.

from kanamycin and is highly effective against gram negative organisms as well as few gram positive organisms[15,16]. It is also effective against gentamycin resistant strains. Although, amikacin is a very effective aminoglycoside against most of the gram negative organisms, it cannot be recommended for all the gram positive organisms. So a combination of antibiotics that can act against various gram negative and gram positive organisms will be useful in field administration. Combined antibiotic therapy is also useful in preventing resistance development and to enhance antibiotic efficacy[15].

Unlike the chemical agents, for a specific detection the bio-threat agents require more time. Till the specific detection, prophylactic drug treatment is required. For gram negative organisms and also as a supportive treatment for gram positive organisms, amikacin is preferable. For viral agents and also for toxins, amikacin can be administered as a supportive therapy. The advantage of amikacin is its safety, stability and can be supplemented with other antibiotics when medical attention arrives. With the use of dual dose autoinjector, amikacin can be given to children and also for pet and farm animals. For biodefence, an antibiotic in an autoinjector is very essential. Amikacin is effective against most of the gram negative organisms. The present *in vitro* study revealed that a combination with either cefozolin or vancomycin can be considered for future development as autoinjector cartridge for the existing autoinjectors.

The usability and tolerability of amikacin administered repeatedly through autoinjectors was earlier studied using

Table 3 . Characteristics of bacterial agents associated with field infections and their drug of choice

Organism	Lethality	Grams reaction	Drugs -first choice	Drugs- other alternatives
Staphylococcus aureus	Low	Gram positive	Penicillin G Methicillin Vancomycin Linezolid	Nafcillin Oxacillin Daptomycin Amikacin[24]
Streptococcus pyogenes	Low	Gram positive	Penicillin G or V	Cephalosporin Erythromycin Azithromycin Clindamycin
Enterococcus faecalis	Low	Gram positive	Penicillin G/ Vamcomycin+ gentamycin	Ampicillin + Sulbactum + Imepenem
Clostridium perfringens	High	Gram positive [Anaerobe]	Metronidazole Gentamycin and amoxicillin	Clindamycin
Clostridium tetani	High	Gram positive [Anaerobe]	Penicillin G, Metronidazole	Clindamycin
Pseudomonas aeruginosa	Low	Gram negative	Ceftazidime Cefepime Amikacin[24]	Aztreonam Meropenem
E. coli	Low	Gram negative	Fluoroquinolones	Amoxycillin+ clavulanic acid Amikacin[15] Cephalosporin, Tigecycline, Polymyxin B
Klebsiella species	Low	Gram negative	Imipenem, Amikacin[15]	Third generation cephalosporin, Cotrimaxazole
Proteus species	Low	Gram negative	Amikacin[15] Cotrimoxazole Imipenem	First generation cephalosporin

various haematological and biochemical parameters in animal models[8,9]. The autoinjectors are self injectable medical devices and are intended for intramuscular or subcutaneous administration. To prove its safety, in the present study the amikacin autoinjector was administered intraperitoneally in rat, where the drug would be delivered with force on the abdominal vital organs. Hence, oxidative stress parameters were carried out in blood. The repeated administration of amikacin both by autoinjector and manual injection did not cause any change in the oxidative stress parameters. Though, autoinjector delivers the drug with a spray effect and with high force, no abnormality was detected in the rats when administered intraperitoneally, showing that autoinjectors are safe.

Conflict of Interest: None

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