Determination of intracellular (neutrophil and monocyte) concentrations of free and liposome encapsulated ampicillin in sheep

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ABSTRACT: In the current study, intracellular (neutrophil and monocyte) concentrations of free and liposome encapsulated ampicillin in sheep were investigated. Free ampicillin (5 mg/kg b.w.) and liposome encapsulated ampicillin (5 mg/kg b.w.) were administered as a bolus intravenous injection to sheep. After the injections, blood samples (5 ml) were collected into tubes from *v. jugularis* at 10, 30, 60 minutes and 2, 4 and 8 hours. Neutrophils and monocytes were isolated, and lysed in distilled water. Ampicillin concentrations were measured by high performance liquid chromatography. The results indicate that liposome encapsulated ampicillin caused the higher intracellular concentrations within neutrophil (ratio of liposome encapsulated ampicillin/free ampicillin; from 1.393 to 5.416) and monocyte (ratio of liposome encapsulated ampicillin/free ampicillin; from 0.973 to 2.906) cells than free ampicillin, and liposome encapsulated ampicillin existed a longer length of time within neutrophil (4 hours) and monocyte (4 hours) cells than free ampicillin (60 minutes), as well. This formulation may be beneficial, in that the treatment of intracellular infections are caused by sensitive bacteria.

Keywords: ampicillin; liposome; neutrophil; monocyte; intracellular concentrations

The penicillins are the most important antibiotic group and presently the drugs of chose for many infections because of their bactericidal effect and low toxicity. Penicillins prevent bacterial cell wall synthesis and disrupts bacterial cell wall integrity. Ampicillin (AP) is a member of the penicillins family, and is widely used in antibacterial chemotherapy. It is used in gonococal urethritis (*Neisseria gonorrhoeae*), upper respiratory infections (*Haemophilus influenzae, Streptococcus pneumoniae* and *Streptococcus pyogenes*) such as sinusitis, tonsillitis, otitis media, pharyngitis, and acute exacerbations of chronic bronchitis, urinary tract infections (Enterobacteriaceae, *Escherichia coli*) and mastitis (Coliform bacteria and *Streptococcus* spp.). In addition to these infections, AP may be used in the treatment of meningitis, salmonella infections and sepsis. Although penicillins are the initial antibiotic chosen in many infections, they poorly penetrate phagocytic cells and have difficulty crossing the serous membranes (Mandell and Petri, 1996; Kaya, 2002; Tras et al., 2005). For this reason, they are not the first chosen drugs in the treatment of intracellular infections caused bacteria susceptible to penicillins.

Liposomes (phospholipid-based vesicles) have been investigated since 1970 as a system for the delivery or targeting of drugs to specific sites in the body. Liposome encapsulated drugs are used in human medicine. Liposome encapsulated antibiotics are especially used in intracellular infections, since liposomes are taken up by reticuloendothelial system cells after intravenous injection. Hence, antibiotics achieve high concentrations into phagocytic

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cells. In addition to this beneficial effect, phagocytosed liposomes are carried to infection area by phagocytes. It was stated that effective treatment of *Listeria monocytogenes* in mice was achieved with liposome encapsulated AP (Fielding, 1991; Gregoriadis and Florence, 1993).

The aim of this study as to determinate intracellular level of free and liposome encapsulated ampicillin after intravenous bolus injections.

MATERIAL AND METHODS

Six healthy sheep (Akkaraman, 15–18 months old, 20–24 kg) were used. All animals were clinically normal and had no received any drugs within 10 days prior to the beginning of the study. The animals were fed a standard diet, with tap water *ad libitum* as well. In the study, free ampicillin (FA, 5 mg/kg b.w.) was initially administered as a bolus intravenous injection. After the withdrawal period, liposome encapsulated ampicillin (LA, 5 mg/kg b.w.) was administered as a bolus intravenous injection (Allen et al., 1998). LA was prepared as described in a previously reported method (Schumacher and Margalit, 1997). The particle size of liposomes was determined with particle sizer (Helos laser diffraction particle sizer, Symphatec GmBH, Germany).

After the injections, blood samples (5 ml) were collected into tubes from *v. jugularis* for the first 10, 30 and 60 minutes, then at 2, 4 and 8 hours. Neutrophil and monocyte was isolated (Kabbur et al., 1991) and lysed in distilled water. After centrifugation, the upper layer was injected into a high performance liquid chromatography (HPLC, Model LC-6A with UV spectrophotometric detector model SPD-6A and data processor model chromatopac CR-6A, Shimadzu Corp., Kyoto, Japan) for determination of AP concentrations (Escudero et al., 1996). In the same supernatant, microprotein levels were measured, as well. AP concentrations were expressed as μg/mg protein.

All values are expressed mean ± SEM. The results were analyzed by Student *t*-test. In all cases, a probability of error of less than 0.05 was selected as the criterion for statistical significance.

RESULTS AND DISCUSSION

Encapsulation ratio, mean size of liposomes, recovery and detection limit of AP were determined as 15 ± 3%, 4.47 ± 1.93 μm, 87 ± 5% and 0.016 μg/ml, respectively. Intracellular concentrations of FA and LA in neutrophil and monocyte cells, and ratio of LA/FA are given Table 1 and 2, respectively.

AP is a widely used antibiotic for the treatment of many infections in veterinary medicine. However, penicillins poorly penetrate phagocytic cells, and have difficulty crossing the serous membranes (Tras et al., 2005). After drugs are encapsulated by liposomes, their pharmacokinetics and efficacious change (Bas et al., 2000; Elmas et al., 2002). In the present study, although LA and FA were injected same dose and route, LA caused the higher intracellular concentrations in neutrophil (ratio of LA/FA; from 1.393 to 5.416) and monocyte (ratio of LA/FA; from 0.973 to 2.906) cells than FA. In addition, LA existed the longest time within neutrophil (4 hours) and monocyte (4 hours) cells than FA (60 minutes). It is stated that after liposomal drugs are injected intravenously, liposomes are rapidly taken up by phagocytic cells (Fielding, 1991), and a similar result was obtained in a previous study where enrofloxacin was researched (Bas et al., 2002). Higher intracellular concentrations of antibiotics enhance the antibacterial activity of phagocytic cells against to intracellular infections when compared to the same free drug dose (Emmen and Storm, 1987; Bas et al., 2000). It was reported that LA and ampicillin-

Table 1. Intracellular concentrations of free ampicillin (FA) and liposomal ampicillin (LA) in neutrophil cells, and ratio of LA/FA (mean ± SEM)

Ampicillin	10 minutes	30 minutes	60 minutes	2 hours	4 hours	8 hours
FA (μg/mg protein)	0.211 ± 0.028[a]	0.071 ± 0.006[a]	0.012 ± 0.007[a]	ND	ND	ND
LA (μg/mg protein)	0.294 ± 0.019[a]	0.156 ± 0.004[b]	0.065 ± 0.011[b]	0.048 ± 0.010	0.013 ± 0.003	ND
LA/FA ratio	1.393	2.197	5.416	–	–	–

[a, b] $P < 0.05$; ND – no determined

Table 2. Intracellular concentrations of free ampicillin (FA) and liposomal ampicillin (LA) in monocyte cells, and ratio of LA/FA (mean ± SEM)

Ampicillin	10 minutes	30 minutes	60 minutes	2 hours	4 hours	8 hours
FA (μg/mg protein)	0.298 ± 0.061[a]	0.099 ± 0.022[a]	0.032 ± 0.013[a]	ND	ND	ND
LA (μg/mg protein(	0.290 ± 0.011[a]	0.148 ± 0.011[b]	0.093 ± 0.007[b]	0.047 ± 0.020	0.014 ± 0.003	ND
LA/FA ratio	0.973	1.494	2.906	–	–	–

[a, b]$P < 0.05$; ND – no determined

bound nanoparticles had higher bactericidal activity in the intracellular infections caused by *Listeria monocytogenes* (Bakker-Woudenberg et al., 1986; Carryn et al., 2003) or experimental salmonellosis (Fattal et al., 1989), listeriosis (Bakker-Woudenberg et al., 1985, 1988; Fattal et al., 1991). On the contrary, it was reported that FA had no good bactericidal activity, nor need higher concentrations to the treatment in the intracellular infections caused by non-typeable *Haemophilus influenzae* (Ahren et al., 2002), non-typhoid *Salmonella* (Chiu et al., 1999) or experimental salmonellosis (Fattal et al., 1989).

As result, LA had the higher intracellular concentration and exited the longest time into phagocytic cells. This formulation may be beneficial in that the treatment of intracellular infections caused by sensitive bacteria and liposome loaded phagocytic cells may be a drug carrier to the infection area.

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