Feasibility of transdermal transport of atenolol by combination of iontophoresis and oleic acid pretreatment

Prospek transpor transdermal atenolol dengan kombinasi iontoforesis dan praperlakuan asam oleat

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Abstract

Atenolol has a low oral bioavailability and a short elimination half-life. Therefore, alternative route and delivery system is important. Transdermal iontophoresis, i.e. a systemic drug delivery via the skin, implementing a low intensity of electrical current, is one attractive candidate. This study evaluated feasibility of atenolol transdermal transport when iontophoresis is applied after enhancer pretreatment. There were 4 formulas prepared; 2 implemented iontophoresis for 3 hours (current density: 0.25 mA/cm^2) while the others did not use iontophoresis. The enhancer was oleic acid (5 or 10% as a mixture in propylene glycol) with duration of pretreatment of one hour. Transport was evaluated in the diffusion studies across the fresh rat skin in a static-vertical diffusion system. Data were analyzed based on the numeric convolution method to obtain simulated Cp profiles as well as AUC of Cp profiles. Based on the simulated Cp, the best transport was achieved in Formula 3, where iontophoresis is performed across the skin, pretreated with 5% oleic acid for one hour. The value of simulated Cp indicated achievement of therapeutics level of atenolol, suggesting the feasibility of the atenolol delivery by iontophoresis.

Key words : atenolol, transdermal, iontophoresis, enhancer

Abstrak

Bioavailabilitas oral yang rendah serta waktu paruh eliminasi yang pendek mendorong pentingnya sistem penghantaran alternatif atenolol. Transdermal iontoforesis, penghantaran obat sistemik melalui kulit menggunakan arus listrik intensitas rendah, merupakan kandidat atraktif. Penelitian ini mengevaluasi prospek penghantaran atenolol melalui kombinasi iontoforesis dengan praperlakuan *enhancer*. Empat formula atenolol dibuat; dua menggunakan iontoforesis (arus listrik 0,25 mA/cm^2, selama 3 jam) dan dua tanpa iontoforesis. *Enhancer* yang digunakan adalah asam oleat (konsentrasi 5 atau 10% dalam propilen glikol) dengan durasi praperlakuan selama 1 Jam. Transpor dievaluasi dengan studi difusi melewati kulit tikus segar pada sel difusi tipe vertikal. Data dianalisis menggunakan metode *numeric convolution* untuk mendapatkan profil simulasi kadar atenolol dalam plasma (Cp) dan area di bawah kurva (AUC) profil simulasi Cp tersebut. Berdasarkan profil Cp simulasi, transpor terbaik diperoleh pada Formula 3 dimana iontoforesis dilakukan melewati kulit yang diberi praperlakuan 5% asam oleat selama 1 Jam. Nilai Cp simulasi mengindikasikan tercapainya konsentrasi terapi atenolol. Hal ini menandakan prospek cerah penghantaran atenolol dengan iontoforesis.

Kata kunci : atenolol, transdermal, iontoforesis, *enhancer.*

Introduction

Atenolol is a selective adrenergic β-1 blocker compound that widely used to treat several cardiovascular malfunctions including hypertension, angina pectoris, tachi-arrhythmic and myocardial infarct. Although has been available in a per-oral dosage form (tablet), atenolol has an intensive first pass metabolism resulting in a relatively low bioavailability (approximately 40%). In addition the compound is also relatively fast eliminated from the body, with a half life of 6 hours (Mason, *et al.,* 1979). Alternative route and delivery system is therefore of great interest. One of good candidate is transdermal iontophoresis, i.e. a systemic drug delivery across the skin, implementing application of a low intensity of electrical current to drive drug molecules transport (Nugroho, 2005).

Several studies have been conducted on transdermal iontophoretics delivery of atenolol. In a recent study, Anroop and coworkers combined iontophoretics method with pro-drug technique to obtain the best transport at a rate of transport of atenolol which could reach the therapeutic level (Anroop, *et al.,* 2009). Another study evaluated the comparison of atenolol transdermal transport in a matrix formulation using oleic acid and iontophoresis. Although the in vivo studies indicated iontophoresis provided lower plasma and skin concentration than the influence of oleic acid pretreatment alone, it was indicated as a good alternative for enhancing the transdermal transport of atenolol (Inal, *et al.,* 2008). Moreover, another research also combined iontophoresis and chemical enhancer as co-treatment to facilitate atenolol transdermal transport. Such combination was reported to be promising, and based on the achieved in vitro atenolol flux, a therapeutically effective concentration could also be attained (Nair, *et al.,* 2009).

In this recent work, we studied feasibility of combination of iontophoresis and skin enhancer pre-treatment, i.e. using oleic acid in propylene glycol, to facilitate transdermal transport of atenolol. The combination has been reported elsewhere to increase transdermal transport of many drugs (Larrucea, *et al.,* 2001; Murakami, *et al.,* 1998). Evaluation was based on the simulated plasma concentration profiles of atenolol, estimated using numeric convolution of the transported amount of atenolol during 3 hours in vitro transport studies.

Methodology

Materials

Atenolol (HCl salt, purity of 99.5%, Calao, Milano Italy) was obtained as a gift from PT Kalbe Farma Jakarta Indonesia. Propylene glycol, oleic acid, mannitol, di-sodium hydrogen phosphate (Na_2HPO_4), Sodium di-hydrogen phosphate (NaH_2PO_4) and sodium chloride (analytical grade, Merck, Darmstadt, Germany) were purchased from PT General Labora Yogyakarta Indonesia. The diffusion studies were performed in a static-vertical diffusion cell (prepared by Department of Physical Engineering *Institut Teknologi Bandung*, Bandung, Indonesia (see Figure 1).

Methods

Rat skin Preparation

Fresh rat skin was obtained directly after sacrificing animals using a lethal dose of ether inhalation. Skin was carefully cleaned from its hair by electric clipping without any damage, wound or scratch on the skin surface. Skin fat was removed by using scalpel. Thereafter, skin was cleaned with aqua pro injection (API) prior to cutting into circular shapes (using a special punch) with a diameter of 2 cm. Skin was then placed onto the diffusion cell, either for a pretreatment studies or directly for a transport study.

Rat Skin pretreatment

After being mounted into diffusion cell, skin was exposed to the enhancer solution; which filled into the donor compartment of the cell. The enhancer consists of oleic acid in propylene glycol based on scheme provided in Table I. Pretreatment was performed for one hour. After the pretreatment, the enhancer mixture was removed from the cell and skin was then cleaned by soaking and spraying with API 5 times. Thereafter skin was ready for an iontophoretic study.

Transport study

After a proper mounting of skin, the cell was then secured by joining and screwing the donor and the acceptor parts together. The donor phase of the system was then filled in with 1 mg/ml atenolol solution (in 5mM citric buffer at pH of 5, containing 4g/l NaCl). The acceptor phase was filled with 0.15M phosphate buffer saline at pH 7.4. Mannitol was added (6 g in 250ml buffer) in the

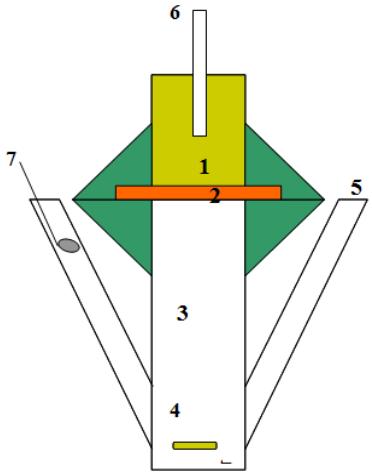

Figure 1. Scheme of static vertical-diffusion cells showing: donor compartment (1), diffusion membrane (rat skin) (2), acceptor compartment (3), magnetic bar (4), sampling port (5), anode (6) and cathode (7).

Tabel I. Formulation design of transdermal iontophoretic transport of atenolol

Formula	Oleic acid (%)	Iontophoresis (mA/cm²)
F1	5	0
F2	10	0
F3	5	0.250
F4	10	0.250

donor solution to balance the tonicity of donor and acceptor media. The iontophoretics study was performed for 3 hours using the iontophoretics power supply (prepared by Department of Electronics, Leiden/Amsterdam Center for Drug Research, Leiden The Netherlands) connected to appropriate electrodes, i.e anode (made of silver plate) and cathode (made of Silver/Silver chloride), dipped in the donor and acceptor medium. Both silver and silver chloride were purchased from Merck, Darmstadt Germany, with a purity of 99.9%. Iontophoretic transport study was performed for 3 hours, during which, samples were collected periodically from the opening column on the acceptor phase side (see Figure 1).

HPLC analyses

Samples were analyses using an HPLC method (HPLC Prominence Shimadzu, Kyoto, Japan) using a LichroCART 250-4 RP 18 (5um) (Merck, Darmstadt Germany) at a flow rate of 0.7ml/minute. The eluent consists of a mixture of 0.05M pH 3.2 acetic buffer and acetonitrile at 70:30 ratio. Atenolol was eluted with at a retention time of 4.6 minute and identified using UV absorption detector (SPD 20A Shimadzu, Kyoto, Japan) at a wavelength of 270nm.

Preparation of atenolol solution

Four formulas of atenolol solution were prepared. Details composition of those, are presented in table I. The studied factors in this research were iontophoretics current (0.25 mA/cm2 and 0 mA/cm2 (without current)) and concentration of oleic acid in propylene glycol (5% and 10%).

Data Analyses

Transport profiles obtained during the studies were analyzed using a numeric convolution method as proposed previously (Qi, *et al.,* 2003; Welin-Berger, *et al.,* 2003), to obtain the predicted plasma concentration profiles, assuming a 1 by 1 in vitro – in vivo correlation. Parameters of disposition of atenolol were obtained from a previously published report (Wan, *et al.,* 1979) Convolution was performed using Kinetica (version 5.0, Thermo Science, USA) implementing the default template provided in this software. Profiles of simulated Cp versus time were then further analyzed to obtain

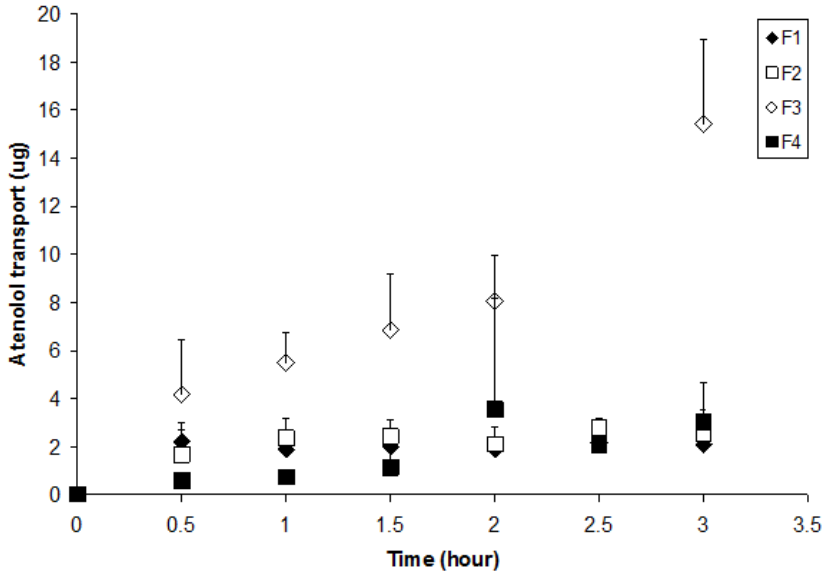

Figure 2. Profiles of atenolol transport in 4 formulas of transdermal transport of atenolol during 3 hours of diffusion studies in vitro in a static vertical diffusion cells across the fresh rat skin. Data are presented as mean + SD (n=3-4).

estimates of area under the curve during 3 hours of transport (AUC). This parameter was used to evaluate the effectiveness of transport on those 4 formulas. Statistical analyses were performed based on ANOVA test followed by a Tukey test ($p<0.05$).

Results and Discussion

Data of atenolol transport under those 4 conditions are presented in Figure 2. There was a relatively similar level of atenolol transport in formula 1, 2 and 4. Levels of transport in formula 3, i.e. iontophoresis at a current level of 0.25mA/cm^2 across skin pretreated with a low concentration of oleic acid (5%), was higher than others ($p<0.05$).

A better insight into the transport level of atenolol in this study was obtained by the results of the numeric convolution method simulating atenolol plasma concentration. Typical examples are presented in Figure 3 where the simulated atenolol concentrations in plasma of Formula 1 and 3 are depicted. Similar to the levels of atenolol transport, Cp levels in Formula 3 was also the highest ($p<0.05$).

Moreover, the estimated area under the curve during 3 hours of the transport (AUC) presented in Figure 4 also show brighter view regarding correlation of the atenolol transport on those 4 formulas. The AUC in Formula 3 was the highest than others ($p<0.05$). This is in contrast to the parameter values in Formula 1, 2 and 4 which were similar one to each other. Such condition is again in agreement with that indicated by the level of atenolol transport. Concerning AUC as one measure of bioavailability, it might suggest that combination of iontophoresis and oleic acid skin pretreatment at a concentration of 5% provided the best transport of atenolol.

Our finding in this study is basically in agreement to what have often been reported that skin pretreatment especially with oleic acid resulted in a significant impairment of the skin barrier integrity. Similarly, Nair and coworkers reported that combination of oleic acid and iontophoresis as co-treatment enhanced atenolol transport significantly. In their study, a range of oleic acid concentration of 1 - 5% w/v was used in a co-treatment method (Nair, *et al.,* 2009). There was a difference in the method of enhancer application, however, as in this studies we implemented a pretreatment style.

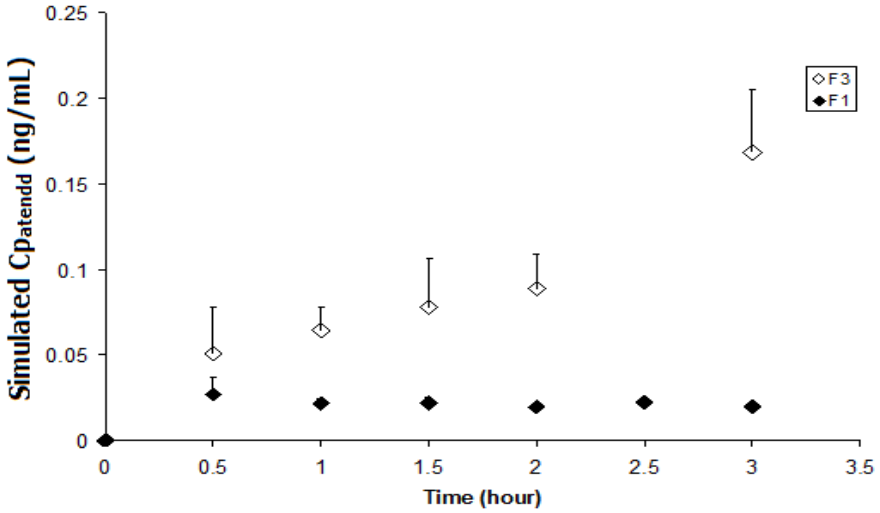

Figure 3. Profiles of simulated atenolol plasma (Cp) based on numeric convolution method of the in vitro transport data of atenolol in Formula 1 dan 3. Data are presented as mean + SD (n=3-4).

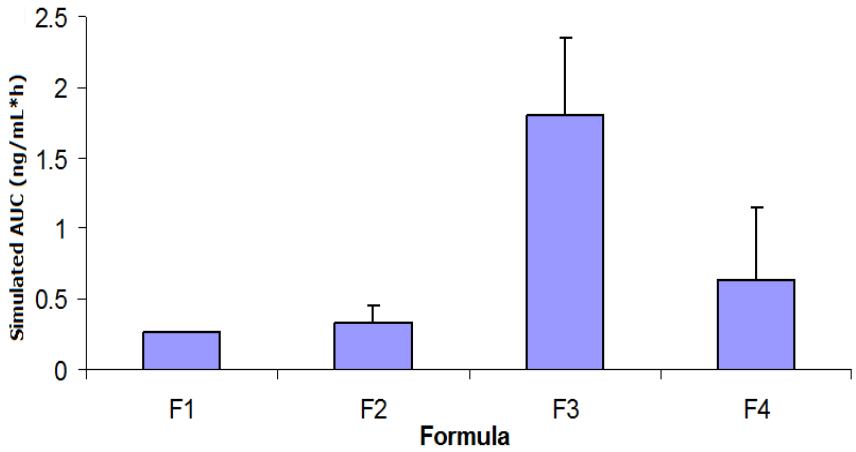

Figure 4. Histogram of AUC of simulated Cp profile during the transdermal transport of atenolol. Data are presented as mean + SD (n=4).

It is interesting that further increase in concentration of oleic acid used for skin pretreatment did not further influence the level of transport of atenolol, either in situation with or without iontophoresis. This is beyond the general expectation that oleic acid could act as enhancer to perturb the integrity of skin barrier (Jiang, *et al.,* 2000; Yu, *et al.,* 2003). In such manner, the higher concentration of oleic acid pretreatment should result in a more permeable skin, thus the higher the transport.

The reasons behind these facts were not fully understood. However, considering its nature as lipid substance, it can be deduced that the presence of oleic acid on the surface of skin after pretreatment could possess problem for the atenolol molecule to pass through the skin. As the study was performed at a pH of 5, due to the pKa of atenolol at around 9.6 (Vogelpoel, *et al.,* 2004), almost 100% of atenolol was present as an ionic state. In such case, the ionic atenolol will have difficulties to

cross the skin with some amount of lipid traces might still be present on skin surface. In addition, the presence of lipid could also reduce the role of skin as a perm-selective membrane which allows drug transport driven by electroosmotic and electrorepulsive force during iontophoresis (Luzardo-Alvarez, *et al.,* 1998). Such hindrance should be more prominent at higher lipid concentration. This explained the reduced iontophoretic atenolol transport at higher oleic acid concentration.

Finally, it is important to evaluate whether therapeutics level could be achieved based on the results of the in vitro studies. The simulated Cp profiles indicated that a maximum value of 0.8ng/mL could be achieved in Formula 3 (with an atenolol donor concentration of 1 mg/mL). This was calculated based on the area of diffusion of the system at a value of 1.88cm^2. In case that a patch at a size of 20cm^2 is prepared, the theoretical concentration of atenolol could be estimated in Equation (1).

Cp = 0.8 ng/mL * (20 cm^2/1.88 cm^2) = 8.5 ng/mL**……(1)**

Considering the solubility of atenolol in water at a value of 26.5 mg/mL (Anonymous, 2009) and assuming that transdermal transport is linearly increased with concentration in donor phase as often reported with several drugs (Nugroho, *et al.,* 2004; Nugroho, *et al.,* 2005), if a concentration of 25mg/ml is used, then the concentration of atenolol in plasma could be estimated in Equation (2).

Cp = 8.5 ng/mL * (25 mg/mL/ 1 mg/mL) = 213 ng/mL=0.213 μg/mL**…..(2)**

According to Puglia dan Bonina, therapeutic window of atenolol is in a range between 0.2 until 0.6 μg/mL (Puglia and Bonina, 2008). Therefore, based on the aforementioned calculation, iontophoresis in combination with oleic acid pretreatment could achieve this important level. This indicated the feasibility of transdermal iontophoretic transport of atenolol to be further developed.

Conclusion

When atenolol is delivered using iontophoresis after a pretreatment of oleic acid at a concentration of 5% (in propylene glycol) with duration of 1 hour, a therapeutics level of the beta-blocker could be achieved, indicating the bright prospect of the formulation to be developed in future.

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