THE INFLUENCE OF PROPYLENE GLYCOL PRE-TREATMENT TO TRANSPORT OF EPIGALLOCATHECIN GALLAT IN GREEN TEA (*Camellia sinensis*, L) EXTRACT ACROSS MICE SKIN, IN VITRO

Nining Sugihartini
Faculty of Pharmacy, Ahmad Dahlan University, Yogyakarta
Email:niningsugihartini@yahoo.com

Abstract

Background. *Epigallocathecin gallat (EGCG) in green tea extract has function as chemopreventive and anticarcinogenesis agents. The previous study reported that EGCG which was given orally will get the first pass metabolism. To avoid this mechanism EGCG could be administrated by transdermal delivery. An enhancer was needed to increase capability EGCG in acrossing the skin layer. The aim of this study was to investigate the influence of enhancer propylene glycol (PG) pre-treatment to EGCG transport across back skin of male mice, in vitro.*

Methods. *Transport of EGCG was performed using vertical type diffusion cells with back skin of male mice as the membrane. Pretreatment was done by membrane soaking with PG at concentrations 0% (control), 5% w/v and 20% w/v for 3 hours. The amount of EGCG which was transported from donor compartment (2% green tea extract in acetate buffer solution pH 4) to acceptor compartment (0,1 M phospate buffer saline at pH 6.2) was analyzed by High Performanca Liquid Chromatography (HPLC) for 26 hours. One way ANOVA was performed to evaluate the cummulative amount and flux of EGCG which was transported across the membrane with 95% significancy.*

Results. *Result of study showed that the mean cumulative amount of EGCG which was transported during 26 hours at concentration of PG 0%, 5% and 20% were 0 µg, 395.26 ± 0.11 µg, 395.29 ± 0.01 µg respectively and the mean of flux were 0 µg/cm^2/s, 8.09 ± 0.01 µg/cm^2/s, 8.10 ± 0.01 µg/cm^2/s respectively. Result of one way Anova showed that there was no significant effect on transport of EGCG between 5% and 20% ($p>0.05$) but there was significantly affect transport of EGCG between 0% and 5% ($p<0.05$).*

Key word : *propylene glycol, epigallocathecin gallate, enhancer*

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INTRODUCTION

Green tea contains of polyphenol which can be used as anticarcinogenesis agent. This agent was known as EGCG. The previous study showed that oral bioavaibility of EGCG was low (Chen et al., 1997). This might be due to an intensive first pass effect and metabolism in the digestive tract or poor absorption in the gastrointestinal tract (Fang *et all*., 2007; Ioannides and Yoxall, 2003).

Based on this condition, it is important to get alternative route of administration. In order to avoid first pass metabolism, transdermal delivery system could be considered. In transdermal drug delivery system, drug must across stratum corneum, the outer-most layer of skin and has function as skin protector. Due to difficulties of drug molecules to cross the stratum corneum enhancer is commonly used (Barry, 1983).

One of the common enhancer is propylene glycol which increases solubility of drug and interacts with protein in stratum corneum (Moolgaard, 1993). Research of Amnuikit et all (2005) showed that PG could increase transdermal delivery of Propanolol HCl. The posible mechanisms of PG as enhancer were : 1) to solve the keratin in stratum corneum (Trommer and Neubert, 2006); 2) to increase the capability of drug to permeate the stratum corneum (Barry, 1991); 3) to increase solubility of drug molecules with protein stratum corneum (Moolgard, 1993) and 4) to hydrate á-keratin of stratum corneum facility the permeation (Walker and Smith, 1995).

The recent study was aimed to examine the influence of propylene glicol pre-treatment to transdermal transport of EGCG in green tea extract across mice skins, in vitro.

METHOD

Material

Oleic acid (pharmaceutical grade from Brataco), EGCG p.a (E Merck), aqua destilata, phosphat buffer saline (Na_2HPO_4 p.a, KH_2PO_4 p.a, KCl p.a, NaCl p.a), Buffer asetat (Na asetat, ammonium asetat, asam asetat glacial), skin of male mice (Balb C).

Preparation of the Enhancer and Donor Solution

The solution of 0%, 5%, and 20% propylene glycol in ethanol was used as enhancer solution. The donor solution was green tea extract 200 mg which was dissolved with 10 ml buffer acetate pH 4.4 to obtain a concentration of 20 mg %.

Preparation of Diffusion Membrane

The two months old mice were sacrificed using lethal dose of aether. The back skin was cut using the surgical scissors. Before the skin was cut in circle, the subcutaneous fats was removed. Then the skin was hydrated in the 0,1 M of PBS (Phosphate Buffer Saline) solution for approximately 30 minutes. Prior to diffusion studies, skin was pretreated using PG solution for 3 hours in the vertical diffusion cell.

The In Vitro Difusion test of EGCG

In vitro diffusion test was performed in vertical difusion cell. The skin was placed between the donor compartment and acceptor, while the stratum corneum directly contact the donor solution. The acceptor compartment was filled in with the PBS solution 0,1 M. at pH 6.2. On the other hand the donor compartment was filled in with green tea extract of 200 mg% in buffer acetate pH 4.4. The acceptor solution was stirred at 50 rpm speed. The sampling was performed during 26 hours at a volume of 3.0 ml in each sample. The amount of EGCG was analysed by an HPLC method.

Analysis

Flux of EGCG transport estimated based on linear regression analysis of the cumulative transport versus time.

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RESULT

Data of EGCG amount transported during the study is present in Table I. Based on these data, the estimated steady state flux is presented in Table I.

Table I. Average amount of cumulative of EGCG in variation of PG

t (hour)	Cumulatif of EGCG/ wide area (μg/cm$^{2)}$		
	PG 5%	PG 20%	Control
	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD
0.5	0	0	0
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	0	0	0
24	273.97 ± 0.01	274.21 ± 0.02	0
25	334.95 ± 0.01	335.18 ± 0.03	0
26	395.26 ± 0.11	395.29 ± 0.01	0

Table II. Amount of flux EGCG at concentration of enhancer PG 5% and 20%

	PG 5%	PG 20%	Control
X±SD	8.09 ± 0.01	8.10 ± 0.01	0

DISCUSSION

This current study showed that the amount of EGCG in aceptor compartment increased for time difusion and started to detect after 24 hours. This finding showed that EGCG needs a long time to across layer of skin as membrane.

As described in the table II, there is significant effect in number of flux betwen control and formulas which get pretreatment with PG. This result shows that PG can be used as enhancer. PG could solubilize drug and function as penetration enhancer. Furthermore, PG could hydrate á-keratin in stratum corneum (Walker and Smith, 1995). Indeed, PG influenced function skin as barrier by increasing solubility of drug and interac with protein in stratum corneum (Moolgaard, 1993). PG was reported both can be used as enhancer and solvent for other enhancers. There is the three phases of PG mechanism, firstly it will penetrate the skin barrier which was not substantially altered, and gradually appeared in the dermis;secondly it will be rapidly distributed in/throughout the dermis, and this rapid distribution was probably due to the alteration of the dermal structure: the penetration enhancing effect of the enhancer was thought to reach maximal; and thirdly, PG was saturated in the dermis

Flux of EGCG at concentration enhancer at 5% and 20% were 8.09 and 8.10, respectively. It means that increasing of consentration of PG did not increase flux significantly. This

condition could be caused the fact that skin has been saturated at concentration 5% so there was no further significant increase at a concentration of 20%.

From this result we suggested to increase the capability of PG as enhancer by combining PG with the other enhancer like terpene or oleic acid. Ota et all. (2003) reported that combination of terpene (5%, w/v) with 30% ethanol, and 20% propylene glycol significantly increased the percutaneous absorption of midazolam in comparison to the control. Similar with this is the oleic acids in combination with PG , pyrrolidones and menthol at low concentrations (5% w/v or less) and PG at 30% w/v . In the other examples, combination of terpenes with propylene glycol/water and Azone with propylene glycol were also reported to increase transport of several drugs .

CONCLUSION

Skin pretreatment with PG 5% caused the significant increase of EGCG transport.

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