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RESEARCH ARTICLE

Combinatorial Approach of Thermosensitive Hydrogels and Solid Microneedles to Improve Transdermal Delivery of Valsartan: an *In Vivo* Proof of Concept Study

Nirmayanti Nirmayanti[1] · Alhidayah Alhidayah[1] · Jessica Theodor Usman[1] · Julika Fajrika Nur[1] · Muh. Nur Amir[1] · Andi Dian Permana[1]

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Abstract

Due to the limitations of oral administration of valsartan, in this study, we aimed to develop thermosensitive hydrogel for sustained transdermal delivery and improved bioavailability of valsartan, which was further improved using solid microneedles. The thermosensitive gel formula was made using Poloxamer 407 and Poloxamer 188 in various ratios. Valsartan thermosensitive gels were evaluated for their gelation temperature, pH values, drug content, spreadability, viscosity, rheological properties, *in vitro* drug release, *in vitro* permeation, and ex vivo permeation. Finally, *in vivo* study was conducted, compared to oral administration. The results presented the formulations showed required characteristic for transdermal administration with desired thermosensitive properties. Based on the permeation test with and without microneedles, it was found that the use of microneedles could affect the permeation of valsartan. Specifically, the increase of microneedles' needle length also increased valsartan permeation. The combination with the highest permeation was produced by 1.55 mm MNs with the amount of drug permeated of 2.27 ± 0.01 mg. Importantly, the transdermal delivery of valsartan using this combination approach could significantly improve the bioavailability of valsartan in *in vivo* study. The concentration of poloxamer was able to affect the properties of the hydrogels, and the use of solid microneedles improved the transdermal delivery of valsartan. *In vivo* studies showed the improvement of the bioavailability of valsartan compared to oral administration, showing the effectiveness of this combination approach.

Keywords solid microneedle · transdermal thermosensitive gel · valsartan

Introduction

Hypertension is a disease that is defined as persistently elevated arterial blood pressure (BP) ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg. Hypertension is one of the primary risk factors for cardiovascular disease [1]. Management of hypertension has become an international health priority [2]. In 2021, as much as approximately 46% of the global adult population was reported to have hypertension [3]. Globally, the prevalence of hypertension increases in exposure to lifestyle risk factors [4]. Hypertension, therewith pre-hypertension, and other hazardously high blood pressure, is liable for 8.5 million deaths from stroke and other vascular diseases worldwide [5].

Valsartan (VALS) (Fig. 1) is a selective angiotensin II type 1 receptor blocker that is used orally. Oral bioavailability (F) of VALS is 10–35% and shows a first-pass effect and low absorption through gastrointestinal. C_{max} and AUC VALS can be diminished by food intake, which may reduce the pharmacological effects [6]. Accordingly, it is critical to develop an alternative delivery route for VALS. It has been previously reported that transdermal administration could be potentially utilized to overcome several issues of the oral route [7–9]. Regarding its properties, VALS has a low molecular weight of 435.5 Da, melting point of 116–117°C, pKa of 4.73, mean biological half-life of 7.5 h and log partition coefficient of 4.5 [10–14]. Additionally, VALS is a candidate that is promising for transdermal drug delivery, and there are no reports about skin irritation caused by VALS [15]

✉ Andi Dian Permana
andi.dian.permana@farmasi.unhas.ac.id

[1] Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia

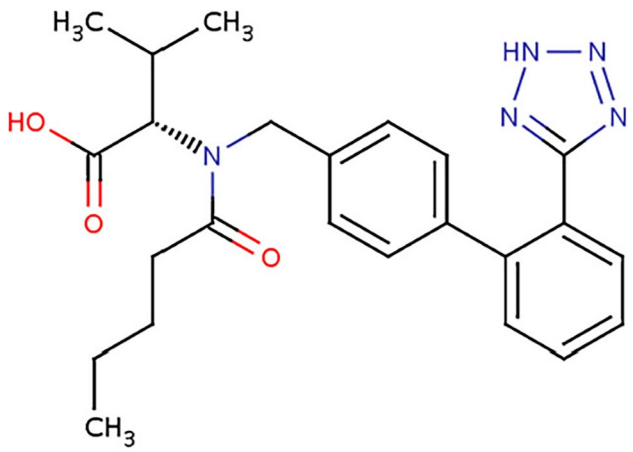

Fig. 1 Chemical structure of valsartan

The major issue in the transdermal delivery system is the presence of *stratum corneum* (SC), the lipophilic layer of skin. It has been reported that VALS has a log partition coefficient of 5.8 [16], showing its lipophilicity which could decrease its ability to reach the dermal circulation for further systemic absorption [17]. One of the strategies adopted to bypass the SC barrier is using solid microneedles (MNs) as physical penetration enhancers attributed to their painless properties and insignificant infection risk at the application site [18]. Among several types of solid MNs, Dermarollers® has been widely used to improve transdermal delivery of numerous active pharmaceutical ingredients [18–20]. MNs as a pretreatment could create micropores in the skin and close by ~ 48 h and would need to be repeated. Therefore, a suitable delivery system is required in order to reduce the application frequency for patient compliance. One of the transdermal delivery systems is thermosensitive gel, the formulation that changes from liquid to gel under an increase in temperature. This approach could potentially be used to reduce the frequency of MN application because it would quickly enter into micropores when applied and change into gel slowly, which would create *in situ* gel depot in the micropores that would continue to deliver drug even if the micropores close [21]

The most frequently used thermosensitive polymer in gel preparations is Poloxamer 407 [22]. However, a Poloxamer 407 solution with more than 20% concentration would form a hydrogel at ambient temperature and reduce the gelation temperature of the preparation to < 25°C so that it can form a gel at ambient temperature [23, 24]. Several studies have shown the limitation of the single use of Poloxamer 407 [25–29], showing the necessity to combine this thermosensitive polymer with another polymer. To overcome this, the addition of another type of poloxamer, Poloxamer 188 has been previously used to modify the gelation temperature of the preparation. It was reported that combining the two types of poloxamer could potentially modulate the gelation temperature of the preparations [30]. To the best of our knowledge, there have been no studies developing VALS in thermosensitive hydrogels for transdermal delivery and its combination with MN delivery system. In this study, we developed thermosensitive hydrogel containing VALS and evaluated their physical and thermosensitive properties. The selected formulation was then combined with solid MNs with different lengths and were evaluated for their permeation profile using a rat skin model. Finally, the bioavailability of VALS using this combination approach was evaluated and compared to oral administration.

Table I Design of VALS Thermosensitive-Gel Formulation

Ingredients % (w/w)	F1	F2	F3	F4	F5
Valsartan	1	1	1	1	1
Ethanol 95%	10	10	10	10	10
Poloxamer 407	17	17	17	18	16
Poloxamer 188	1	3	5	2	4
Distilled water	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100

Materials and Methods

Materials

Valsartan (Purity: > 98.0%) was obtained from Tokyo Chemical Industry (Tokyo, Japan). Poloxamers 407 and 188 were kindly provided by BASF Indonesia, Jakarta). Solid microneedles (Dermarollers®) were purchased from SQY® (Guangdong, China). Other compounds used in this study were of analytical grade.

Design of Formulation

The poloxamer solution was prepared using the cold method by slowly adding the poloxamer combination that was weighed accurately into cold distilled water (4°C). Table I shows the composition of the formulation. The solution was further stirred constantly to form a poloxamer solution. The poloxamer solution was then stored in the refrigerator overnight until a clear solution was formed. Following this, VALS was dissolved in ethanol 95% and mixed in an already-prepared poloxamer solution at 4°C [15, 17].

Evaluation of Physical Properties

Gelation Temperature

Gelation temperature was determined by placing 2 mL of each formula into a test tube [24, 31]. The test tube was

put into a water bath at 20°C. Furthermore, the test tube was rotated at 90° every 1°C increase in temperature until the temperature reached 65°C. The gelation temperature recorded was the temperature at which the gel did not move when the test tube was rotated. The test was run in triplicate.

pH Determination

The pH of the VALS thermosensitive gel was measured using a digital pH meter, and the test was performed in triplicate [32].

Drug Content

VALS thermosensitive gel (0.1 mL), equal to 10 mg of VALS, was pipetted out. It was diluted with ethanol, and the absorbance of the mixture was measured at 230.2 nm with a UV–Vis spectrophotometer (Dynamica, HALO XB-10). The drug content was calculated against the absorbance of the control VALS solution at 230.2 nm. [33]. The concentration of VALS was determined by plotting the absorbance values to the calibration curve prepared from the standard solution of VALS in the concentration range of 0.5–32 µg/mL.

Spreadability

The spreadabality of VALS thermosensitive gel was determined at gelling state by placing an amount of 0.5 g gel in a 2-cm-diameter circle premarked on a glass piece over a second glass plate. A weight of 500 g was rested on the top of the glass piece for 5 min. The diameter increased appropriately to the preparation's spreading was recorded in triplicate [15].

Viscosity and Rheological Properties

The viscosity of VALS thermosensitive gel was determined using a viscometer (Brookfield, USA) at various temperatures (4 ± 0.1°C, 25 ± 0.1°C, and 35 ± 0.1°C). The study was performed by placing each formulation on the lower plate of the apparatus, and the viscometer was run using spindle number 63 at 60 rpm. The rheological behavior was determined by calculated viscosity against the various velocities (rpm) of VALS thermosensitive gel [14, 15].

In Vitro Release Study

The *in vitro* release of VALS from thermosensitive gels was measured by dissolution model without membrane, as previously described [17], with minor modifications. The amount of 5 g VALS thermosensitive gel was taken, put in a glass vial, and equilibrated to the gelation temperature at 37 ± 1°C. Following this, 2.5 mL of PBS was poured smoothly on the upper gels without interfering with the gel surface. Then, 1 mL was collected at regular time intervals (0.5 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, and 24 h) for release quantification. For maintaining the sink condition, fresh PBS medium (1 mL) was added to the glass vials each time. The absorbance of samples was measured using a UV–Vis spectrophotometer (Dynamica, HALO XB-10, UK) at 231.4 nm, and the amount of VALS released was calculated. The release study of free VALS was also evaluated as a control. The concentration of VALS was determined by plotting the absorbance values to the calibration curve prepared from the standard solution of VALS in the concentration range of 0.5–32 µg/mL.

In Vitro Permeation Study

Franz diffusion cell was used to assess the *in vitro* permeation of VALS. The cellophane membrane was used as a membrane for the study. The receptor compartment was filled with PBS (pH 7.4) and magnetic bar was placed inside for stirring. The membrane was placed in between the receptor and the donor compartment. The cell was held at 37 ± 1°C and agitated by a magnetic stirrer at 100 rpm. An amount of 1-g gel preparation was placed in the donor compartment. About 1 mL sample was taken at regular time intervals. After each time, an equal volume of fresh PBS (pH 7.4) heated to 37 ± 1°C was added to the receptor compartment to keep the sink conditions. The sample absorbance was measured using a UV–Vis spectrophotometer (Dynamica, HALO XB-10, UK) at 231.4 nm [18, 33]. The *in vitro* permeation study of free VALS was also evaluated as a control. The concentration of VALS permeating was calculated by plotting the absorbance values to the calibration curve prepared from the standard solution of VALS in the concentration range of 0.5–32 µg/mL.

Ex Vivo Permeation Study

Similarly, the Franz diffusion cell was also used to investigate the ex vivo permeation study [20, 35, 36]. The abdominal skin of female Sprague–Dawley rats was shaven and used as a biological membrane for the study. Initially, the hair of the rats was shaved, and the hair removal cream was applied to the shaved area. The rats were euthanized using ether and were sacrificed. Following this, the skins were collected. The skin was washed with distilled water and equilibrated in PBS before the experiment. The ex vivo experiment was approved by the Health Ethical Committee at the Faculty of Medicine, Hasanuddin University, Indonesia. The receptor compartment was filled with 10 mL of PBS (pH 7.4) and magnetic bead for stirring. In an attempt to evaluate the effect of solid MN treatment on the penetration through the skin, the skin sample was pretreated with a Dermaroller®

with various lengths (0.5 mm, 1 mm, and 1.5 mm) before it was placed onto the donor compartment. The skin (surface area of 1.77 cm^2) was placed in between the receptor and the donor compartment. The cell was maintained at 37 ± 1°C and agitated by a magnetic stirrer at 200 rpm. An amount of 1 g of gel was placed in the donor compartment. About 1 mL sample was withdrawn at regular intervals (0.5 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, and 24 h) and replaced by 1 mL fresh PBS (pH 7.4) heated to 37 ± 1°C to keep the sink conditions. The sample absorbance was assessed using a UV–Vis spectrophotometer (Dynamica, HALO XB-10) at 231.4 nm. The concentration of VALS was determined by plotting the absorbance values to the calibration curve prepared from the standard solution of VALS in the concentration range of 0.5–32 µg/mL.

In Vivo Study

The *in vivo* investigation was conducted to investigate the penetrability of VALS from thermosensitive hydrogels after the application of solid MNs. The study was performed on healthy male Wistar rats weighing 213.08 ± 14.17 g. All the animals were acclimatized for 1 week in the conditions of the laboratory. The *in vivo* experiment was approved by the Health Ethical Committee at the Faculty of Medicine, Hasanuddin University, Indonesia. There were three groups in this study: the first group received thermosensitive gels containing VALS after the administration of solid MNs; the second group received thermosensitive gels containing VALS without the administration of solid MNs; and the third group received oral administration of VALS. All groups received VALS with a dose of 10 mg/kg of body weight. At predetermined interval times (1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, and 24 h), the blood was collected, and the plasma samples were separated for further analysis.

Sample Preparation, Extraction and Analysis

VALS was extracted from plasma using a simple extraction method using methanol. Briefly, 750 µL of methanol was used to extract VALS from 100 µL of plasma. The extraction was performed by mixing methanol and plasma samples using a vortex for 20 min. Following this, the samples were centrifuged at 12,000 rpm for 15 min, and the supernatant was collected and dried for 3 h, obtaining dry residue. The quantification of VALS extracted from plasma samples was performed using bromophenol blue method, as reported previously [37], with slight modifications. The residue was reconstituted using 450 µL methanol and mixed with 50 µL bromophenol blue solution in methanol (1.3×10^{-3} M). The concentration of VALS was determined by measuring the absorbance of the solution at 424 nm using a UV–Vis spectrophotometer (Shimadzu Co., Ltd., Tokyo, Japan). The concentration of VALS in plasma samples was determined by plotting the absorbance values to the calibration curve prepared from the standard solution of VALS in plasma in the concentration range of 1–64 µg/mL. Afterwards, the curves consisting of the VALS concentration and time of application were prepared. The pharmacokinetic profiles were calculated using PK Solver [38].

Statistical Analysis

All the data were presented as means ± standard deviation (SD) of the mean. SD of the results was measured using Microsoft® Excel® 2010 (Microsoft Corporation, Redmond, USA). The statistical analysis was performed using GraphPad Prism® version 5.0.3 (GraphPad Software, San Diego, California, USA). The $p < 0.05$ indicated a significant difference in all cases.

Result and Discussion

VALS Thermosensitive Gel Formulation

In this study, a thermosensitive transdermal gel formulation of VALS was carried out, and several physical characteristics, release profile, and drug permeation in rat skin were performed in an ex vivo study. VALS thermosensitive gel was made in five different formulas containing a combination of Poloxamer 407 and Poloxamer 188 as thermosensitive polymers. The representative image of the resulting preparation is depicted in Fig. 2. The results of organoleptic observations showed that all the thermosensitive gel preparations were slightly clear with a smell of alcohol. This was in accordance with previous research, showing that gels should appear transparent [39]. In addition, the resulting preparation of thermosensitive gel was in liquid form at room temperature and gel form when the gelation temperature was reached.

Evaluation of Physical Properties

Effect of Poloxamer Concentration on Gelation Temperature

Gelation temperature testing was carried out to obtain the temperature required by VALS thermosensitive transdermal gel to transform into gel form. The thermosensitive transdermal gel should have a gelation temperature similar to the physiological skin temperature of 32 ± 1°C. Hence, when the transdermal gel is applied, the preparation would become gel on the skin [40]. The gelation temperatures of VALS thermosensitive transdermal gel are exhibited in Fig. 2. The test results show that the gelation temperatures of all formulations obtained were in the range of 27.67– 40.67°C. The

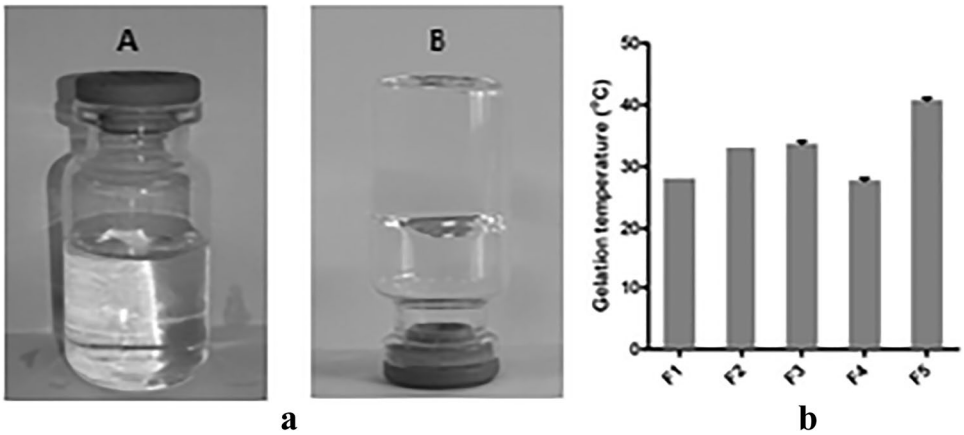

Fig. 2 **a** VALS thermosensitive gel a in room temperature **b** in body temperature; **b** gelation temperature of VALS thermosensitive gel (mean ± SD, $n = 3$)

results showed that F2 and F3 had gelation temperatures that were most similar to the physiological skin temperature. Thermosensitive gels with the same concentration of Poloxamer 407 and Poloxamer 188 showed that gelation temperature increased when the concentration of Poloxamer 188 was raised. On the other hand, increasing the concentration of Poloxamer 407 decreased the gelation temperature of the thermosensitive gel [41]. The difference in gelation temperature of each formula was due to differences in the hydrophilic and hydrophobic structure of poly(propylene oxide) (PPO) and poly(ethylene oxide) (PEO) from Poloxamer 407 and Poloxamer 188 [28]. The basic structure of poloxamer consists of one PPO block flanked by two PEO blocks with different block lengths and produces various variations of poloxamers [42]. The hydrophobic and hydrophilic ratios of Poloxamer 407 and Poloxamer 188 are 3:7 and 2:8, which makes Poloxamer 407 more hydrophobic. The PPO hydrophobic block would cause a decrease in gelation temperature, while the PEO hydrophilic block would increase the gelation temperature. Thus, the greater the PPO ratio of the VALS gel formulation, the lower the gelation temperature of the preparation [34]. The data obtained showed that the gelation temperature of each formula was significantly different ($p < 0.05$).

Effect of Poloxamer on pH, Drug Content, and Spreadability

The pH test was carried out to investigate whether the skin could well tolerate the pH of the transdermal gel preparation. Generally, skin can tolerate the administration of the topical preparation with the pH values ranging from 4 to 7 [43]. Preparations with a pH outside this range could potentially cause problems, such as irritation, itching, or burning of the skin [44]. As shown in Table II, all the measured pHs were found to be in the range. Statistical analysis showed that the poloxamer concentration increased the pH of the preparation but was not significant ($p > 0.05$) [45]. Meanwhile, the results obtained in Table II reveal that the drug

Table II The Result of pH, Drug Content and Spreadability Test of VALS Thermosensitive Gel (Means ± SD., $n = 3$)

Formula	pH	Drug content (%)	Spreadability (cm^2)
F2	4.05 ± 0.0	99.19 ± 0.03	7.15 ± 0.2
F3	4.15 ± 0.0	97.11 ± 0.02	0.2

content in both formulas was found to meet the requirements for drug content standards within 95–105% [46]. Based on these data, it was known that the content of each drug in both formulations was not statistically different ($p > 0.05$). Accordingly, it can be seen that the difference in the concentration of poloxamer did not significantly influence the drug content in the preparation and also shows that the gel preparation had uniform drug content that ensured a more accurate dosage when given to the patient. Additionally, the spreadability of all formulations shown in Table II indicates that F2 had a higher spreadability than F3. This was because F3 contained poloxamer with a higher concentration, which made the preparation's viscosity higher, and the spreadability decreased. Based on these data, it was known that the spreadability of the VALS gel in the two formulations has a significant difference ($p < 0.05$) due to the difference in the concentration of poloxamer in these hydrogels. Spreadability is one of the most important physical characteristics in a semi-solid preparation because it is responsible for administering the dose to the target and also for the ease of application [47].

Effect of Poloxamer Concentration on Viscosity and Rheological Properties

Viscosity is the most crucial rheological parameter to indicate the flow of preparation [48]. The results are depicted in Fig. 3, indicating that the viscosity of F3 was higher than that of F2 due to the fact that F3 contained a higher concentration of poloxamer combination when compared to F2. The

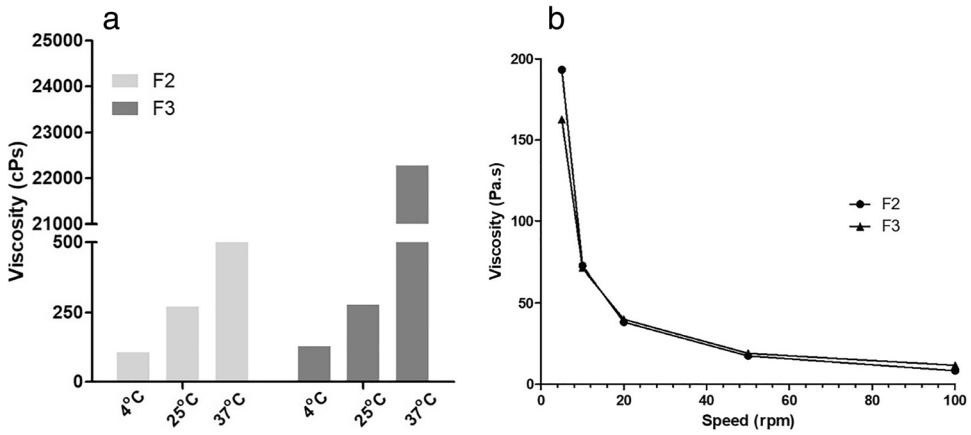

Fig. 3 **a** Viscosity of VALS thermosensitive gel in different temperatures; **b** rheology of VALS thermosensitive gel with variety of spindle rotation (rpm) (means ± SD., $n = 3$)

cross-linking of the hydrophobic groups of the poloxamer affects the gel strength. The fewer cross-links formed, the softer the gel, and the lower the viscosity of the gel [49]. From these data, it was also found that an increase in temperature can increase the viscosity of the preparation, especially when at body temperature, which indicates that at cold temperatures and room temperature, the preparation was still in liquid form. Furthermore, the preparation turned into a gel when it reached body temperature. Importantly, rheology studies the flow of a liquid preparation, especially non-Newtonian liquids exhibited by materials such as polymers. This long chain molecule most often exhibits a pseudoplastic type of flow, showing a viscosity decrease when the force increases. In the gel rheology test, the following results are shown in Fig. 3. These results indicate that both formulas F2 and F3 had a pseudoplastic flow type because there was a decrease in viscosity and an increase in spindle speed during measurement. Based on these data, it was observed that the poloxamer concentration significantly affected the viscosity of the formulas ($p < 0.05$).

Effect of Poloxamer Concentration in *In Vitro* Release and Permeation of VALS

The incorporation of VALS into thermosensitive gel could potentially sustain the release and permeation of VALS. The following graph presented in Fig. 4a shows that the amount of drug released from the preparation after 24 h from F2 was 47.35 ± 0.23 mg, and F3 was 49.36 ± 0.24 mg. On the other hand, 49.36 ± 0.27 mg of VALS released only after 2 h in case of free VALS. This indicated the sustained release behavior was achieved following the incorporation of VALS into thermosensitive hydrogels. Moreover, Fig. 4b shows the amount of *in vitro* drug permeated from the preparation after 24 h. VALS permeated from F2 and F3 were 3.05 ± 0.21 mg and 3.65 ± 0.25 mg, respectively. Released without controlled release manner, 3.45 ± 0.19 mg of VALS permeated within 3 h. Performed in ex vivo study, the amount of VALS permeated from the formulation after 24 h were 1.35 ± 0.05 mg and 1.64 ± 0.06 mg for F2 and F3, respectively. With respect to the permeation profile of free VALS, the sustained release pattern was not also observed, showing the amount of 1.46 ± 0.02 mg of VALS permeated after 3 h in ex vivo permeation studies. Analyzed statistically, the release and permeation profiles of VALS in *in vitro* and *ex vivo* studies from both formulations were significantly different ($p < 0.05$). The difference in the release profile might be due to the concentration of poloxamer used. The increase in the concentration of Poloxamer 188 could increase drug release from the preparation [50]. Poloxamer 188 has a relatively shorter chain and a smaller molar ratio or tendency to interact with water. Thus, it could potentially increase the water molecules around the hydrophilic PPO block in Poloxamer 407, which made the preparation tend to be eroded, and the drug could be more easily released from the preparation. According to the results, F3 was selected for further studies.

Effect of MNs in *Ex Vivo* Permeation of VALS

Finally, to improve the transdermal delivery of VALS from thermosensitive hydrogels, solid MNs were used. In the permeation profile investigation using MNs, three types of Dermaroller® with different needle lengths (0.5 mm, 1.0 mm, and 1.5 mm) were used to see the effect of MN needle length on VALS permeation in F3, which showed better physical characteristics, release profile, i*n vitro* permeation, and ex vivo permeation. Figure 5a shows the amount of permeated VALS for up to 24 h. In the test without MNs, VALS permeated was found to be only 1.64 ± 0.05 mg. On the other side, preparations combined with MNs showed an increase in the amount of drug permeated. The combination with 0.5 mm MNs showed the amount of drug permeated of 2.32 ± 0.01 mg; the combination with 1.0 mm MNs was 2.65 ± 0.02 mg; and the combination with 1.5 mm MNs was 3.43 ± 0.02 mg. These results showed that the use of MNs could successfully

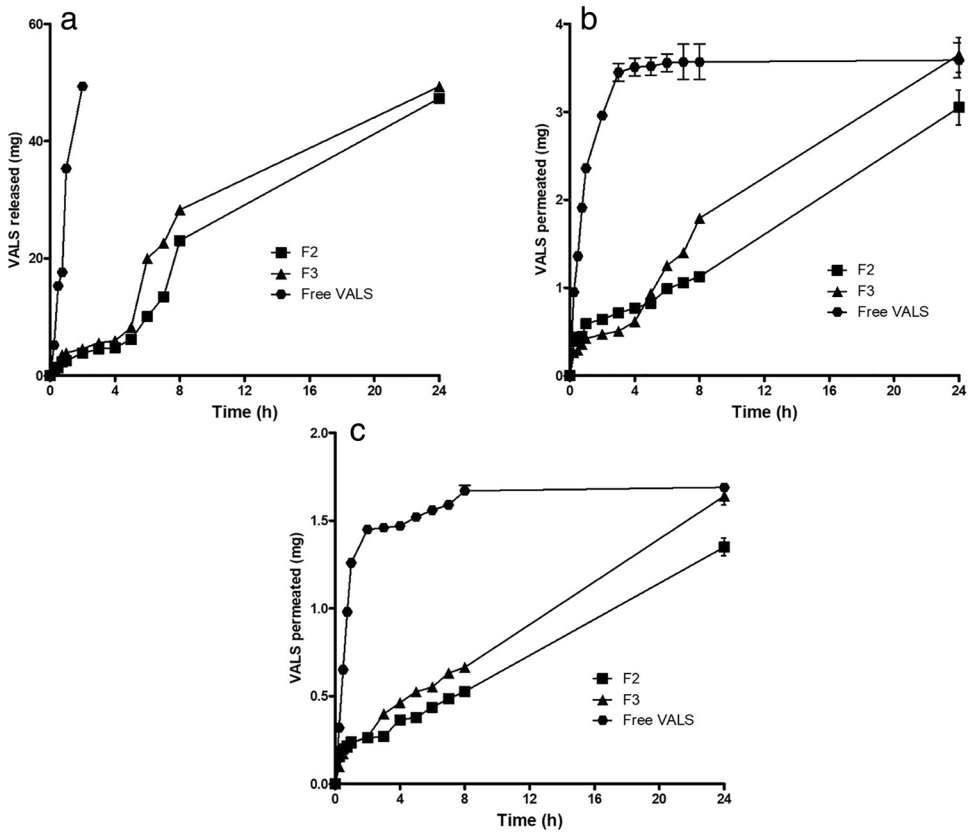

Fig. 4 The result of *in vitro* release of VALS thermosensitive gel **a**; *in vitro* permeation of VALS thermosensitive gel **b**; and ex vivo permeation of VALS thermosensitive gel **c** (means ± SD., $n = 3$)

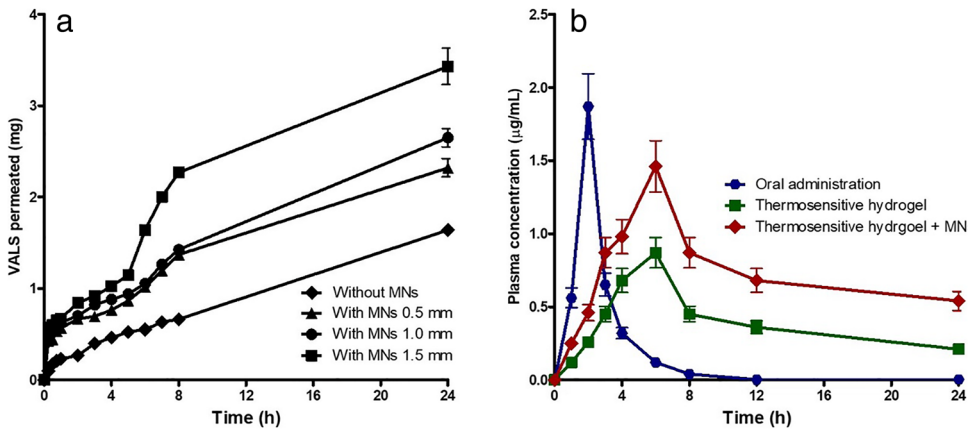

Fig. 5 The result ex vivo permeation of VALS from thermosensitive hydrogel F3 with and without MNs a; mean plasma concentration of VALS at several interval times following different administration routes b (means ± SD., $n = 3$)

increase the permeation of transdermal preparations because it could form micropores on the skin. Therefore, the drug would easily pass through the SC, the skin's largest barrier component. Furthermore, it was observed that the longer the MN needle, the greater the amount of drug permeated [21]. The results showed a significant increase in the amount of permeated VALS ($p < 0.05$) when VALS was combined with MNs so that it could potentially increase the amount of VALS that entered the body.

In Vivo Study

As previously discussed, this study was designed to overcome the bioavailability issue of VALS when administered through oral administration. In this study, the pharmacokinetic profiles of VALS delivered from the combination of thermosensitive gel and solid MNs were compared to the administration of thermosensitive gel without solid MNs and the conventional oral administration. The plasma

Table III Pharmacokinetic Parameters of VALS Following the Oral Administration, the Transdermal Delivery of Thermosensitive Hydrogel and the Combination of Hydrogel With MNs (Means ± SD., $n = 3$)

Parameter	Unit	Oral	Thermosensitive hydrogel	Thermosensitive hydrogel + MNs
$t1/2$	h	2.52 ± 0.32	14.75 ± 2.11	25.33 ± 3.09
T_{max}	h	2.00	6.00	6.00
C_{max}	µg/mL	1.87 ± 0.23	0.87 ± 0.98	1.46 ± 0.21
AUC 0-t	µg/ML × h	3.97 ± 0.45	9.08 ± 1.02	17.26 ± 1.98
AUC 0-inf	µg/mL × h	3.98 ± 0.51	13.55 ± 1.65	37.00 ± 4.56
MRT 0-inf	h	2.97 ± 0.35	21.73 ± 2.33	37.46 ± 4.87

concentrations of VALS following three different administrations are depicted in Fig. 5b. Moreover, several pharmacokinetic parameters were evaluated, such as $t1/2$, T_{max}, C_{max}, AUC, and MRT, as depicted in Table III. The $t1/2$ is the time where the concentration of the drugs in the body decreases by half of the initial dose. C_{max} represents the highest concentration of the administered drugs in the body. Importantly, AUC values are utilized to assess the total drug exposure in the body during the evaluation time. The MRT represents the average time of the drugs administered residing in the body [51]. Following oral administration, it was found that $t1/2$, T_{max}, and C_{max} values of VALS were 2.52 ± 0.32 h, 2 h, and 1.87 ± 0.23 µg/mL. These values were also similarly observed in a previous study performing pharmacokinetic determination of VALS using a similar dose with VALS dose used in our study [43]. After the transdermal delivery using thermosensitive hydrogel, the values were 14.75 ± 2.11 h, 6 h, and 0.87 ± 0.98 µg/mL for $t1/2$, T_{max}, and C_{max}, respectively. Importantly, with the combination with MNs, that $t1/2$, T_{max}, and C_{max} values of VALS were 25.33 ± 3.09 h, 6 h, and 1.46 ± 0.21 µg/mL. The results showed that compared to oral administration, transdermal delivery could improve the bioavailability of VALS, indicated by the improvement of AUC values. The highest AUC value was achieved after the combination of thermosensitive hydrogel with MNs, showing the value of 17.26 ± 1.98 µg/mL × h. This value was significantly higher ($p < 0.05$), compared to AUC values of oral administration and thermosensitive hydrogel groups. Moreover, the residence time of VALS was also significantly improved through transdermal delivery, showing the controlled release manner of VALS. As discussed previously, the pores created by MNs would allow the effective permeation of the thermosensitive hydrogels. Due to the thermosensitive properties, the transformation of the formulation into gel in the body temperature would be beneficial to sustain the release of VALS to the systemic circulation from the skin. Accordingly, the combination of thermosensitive and MNs offers two main benefits. First, it could improve the bioavailability of VALS. Second, this approach could sustain the *in vivo* release of VALS. This could potentially reduce the administration frequency of the treatment, leading the patient compliance during the hypertension therapy.

Overall, the results obtained in this study served as a proof of concept, showing that the permeation of VALS through the skin from thermosensitive hydrogel could potentially be improved using solid MNs. This could be beneficial in the treatment of hypertension. However, *in vivo* animal works using hypertension model should now be carried out to investigate the effectiveness of this combination approach. In addition, dose determination, drug toxicity, and skin irritation studies should also be considered.

Conclusion

This study investigated the potency of a transdermal delivery system to enhance the VALS permeated through the skin layer using poloxamer-based thermosensitive gel. According on the findings presented in this study, it was shown that VALS thermosensitive gel was successfully prepared using combination of Poloxamer 188 and Poloxamer 407, where concentration of poloxamer affected the physical properties of thermosensitive gel. Furthermore, the combination of VALS transdermal thermosensitive gel and solid MNs enhanced the permeation of VALS in ex vivo rat skin and improved the bioavailability of VALS in *in vivo* study. The major advancement of the combinatorial delivery system we have presented in this work led to higher permeation through the skin that could improve the efficiency of hypertension therapy, hypothetically. Building from these stated results, further studies including *in vivo* efficacy studies should be carried out to completely investigate the therapeutic efficacy of this approach in hypertension animal models.

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Declarations

Ethics Approval We have no ethical issue to declare.

Competing Interests The authors declare no competing interests.

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