ISSN: 0976-822X

Available online on www.ijcpr.com

International Journal of Current Pharmaceutical Review and Research 2022; 14(4); 60-68
Original Research Article

The Anticonvulsant Activity of Mucoadhesive Buccal Films of Sodium Valproate in Pentylenetetrazole-Induced Seizure Rat Model

Iwan Setiawan[1,2], Anas Subarnas[1], Yoga Windhu Wardhana[1]

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, West Java, Indonesia
[2]Sekolah Tinggi Ilmu Kesehatan Nasional, Sukoharjo, Center of Java, Indonesia

Received: 20-11-2022 / Revised: 01-12-2022 / Accepted: 20-12-2022
Corresponding author: Iwan Setiawan
Conflict of interest: Nil

Abstract

Buccal preparations are currently gaining interest in the study of drug delivery systems. These preparations can adhere to mucosal surfaces, hydrate, and deliver the drug across the buccal membrane. Based on the Biopharmaceutics Drug Disposition Classification System (BDDCS), the drug compound Sodium valproate Sodium valproate belongs to class 1 which has excellent solubility and permeability. Therefore, by developing into a mucoadhesive buccal dosage form, a faster onset of action will be obtained, increased bioavailability and ease of use. In partial epilepsy, bipolar disorder (psychosis), and migraine therapy, sodium valproate is the first-line anticonvulsant. Therefore, this study aims to develop sodium valproate into a mucoadhesive buccal film dosage form using the solvent casting method. The preparations were then tested for antiepileptic activity, and the pharmacokinetic profile analysis was conducted in test animals induced with the pentylenetetrazole compound using a Post Randomized Controlled Group Design. The results showed an anti-epileptic activity with a longer duration of onset, shorter duration of seizures, and lower seizure frequency when compared to the experimental animal group administered sodium valproate tablets. The pharmacokinetic profile of sodium valproate included absorption parameters of Ka, Tmax, Cmax, Vd, T1/2 el, and Kel of 0.0033 Hr-1, 3.93 Hr-1, 4.55 mg/L, 3.03 L, 21.8 minutes, and 0.0053/minute, respectively. Based on the results, sodium valproate mucoadhesive buccal film preparation can be developed into an anti-epileptic preparation with a delayed release profile, and a relatively fast onset, making it a breakthrough for antiepileptic therapy.

Keywords: Mucoadhesive buccal film, Natrium valproate, Pentylenetetrazole, Anti epilepsy, Pharmacokinetic profile

This is an Open Access article that uses a fund-ing model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

INTRODUCTION

Epilepsy is defined as a disruption in the balance between cerebral excitation and inhibition, resulting in the cessation of uncontrolled excitation. Furthermore, the balance of Gamma Amino Butyric Acid (GABA) and glutamate, which are the primary inhibitory and excitatory neurotransmitters in the brain, respectively, determine normal brain function. This causes the brain tissue to become hyper-excited when excitation exceeds inhibition, resulting in a low seizure threshold. If the imbalance is large enough, seizures can occur, leading to epilepsy.[1]

Sodium valproate1 has a broad spectrum of anticonvulsant activity but is structurally unrelated to conventional

antiepileptic drugs. Its proposed mode of action is mediated by effects on the GABA function in the brain. Another known mechanism of action involves inhibiting the succinic semialdehyde dehydrogenase enzyme, which then inhibits GABA metabolism by GABA transaminase, resulting in increased GABAergic neurotransmission.[2]

Elevations in cerebellar GABA, as well as the concomitant reductions in cyclic guanosine monophosphate levels, occur in animals at dose levels that are unlikely to be achieved during the treatment of epileptic patients. Sodium valproate is an antiepileptic drug that inhibits absence, partial, and tonic-clonic seizures by increasing Na+ channel inactivation, thereby decreasing the ability of nerves to conduct electrical charges[3]. The success of sodium valproate therapy in treating epilepsy remains elusive. The buccal drug delivery route was hypothesized to be a promising approach for administrating sodium valproate because it offers rapid and direct delivery into the systemic circulation, bypassing any degradation in the gastrointestinal tract and first-pass metabolism in the liver. This elevates the bioavailability of the drug and provides a steady-state plasma drug level, which increases the therapeutic efficiency.[4]

Pentylenetetrazole (PTZ) is a selective antagonist of the GABAA chloride ionophore complex receptor and PTZ-induced seizures are one of the gold-standard mouse models for the rapid evaluation of novel anticonvulsants.[5] This delivery route is also preferred for the targeted, controlled, and sustained release of drug molecules. Considering the significance of the buccal route, this study aims to assess the feasibility of delivering sodium valproate through the oral mucosa by formulating buccoadhesive films. These films can increase the onset of action, prolong drug release, and improve bioavailability. The prepared films were characterized, and the dosage forms were evaluated in-vitro and in-vivo. Additionally, the effect of different polymers on the mucoadhesion time of buccal films and factors influencing drug release from the film were examined.

METHODOLOGY

Material

The tools used included a Shimadzu 1280 Double Beam UV/Vis Spectrophotometer, an Analytical Scale (OHAUS Pioneer) with a sensitivity of 0.0001 g, a 50–1000 L Micro Pipette (SOCOREX), a centrifuge (Effendorf MiniSpin), glass tools such as measuring flasks, beakers, measuring cups and test tubes, and other laboratory support equipment. The materials used in this experiment include Sodium valproate obtained from PT. Otto Pharmaceutical Lab, which had been formulated into Buccal mucoadhesive dosage forms, Depacote® (AbbVie Ltd, Imported and packed by PT. Abbott Indonesia), Chitosan from Sigma Aldrich, Sodium Carboxymethyl cellulose (SCMC), Propylene glycol, Pharmacoat 604, Acetic acid, Na EDTA, Ketamine, Wistar white rats obtained from the Pharmacy Study Program of the National College of Health Sciences, and PTZ from Sigma Aldrich.

Animals

In this study, 18 male Wistar rats were used and divided into four groups of six each. Group I rats received 0.5% suspension SCMC as Negative Control, while Group II rats received intraperitoneal (IP) sodium valproate at a dose of 100 mg/KgBB. Furthermore, Group III rats were treated with per oral sodium valproate tablets at a dose of 4.5 mg, while group IV were treated with mucoadhesive buccal film sodium valproate at a dose of 4.5 mg. The animal experiments were approved by the Health Research Ethical Committee for Animal Experimentation, Faculty of Medicine, Muhammadiyah University of Surakarta (No. 3638/A.1/KEPK-FKUMS/VII/2021). The White male

Wistar mice used in this study weighing 120 ± 3 g and aged 1 ± 0.3 years were obtained from the Pharmacy Studies Program of the National College of Health Sciences and were found to be healthy by a general clinical examination, complete blood count, and serum biochemistry panel. The mice were housed in individual pens for one week before the study for acclimatization. They were then administered concentrated feed containing 2.750 kcal/kg metabolic energy, 12% crude protein, 88% dry matter, 12% crude ber, 25 mg/kg vitamin E, 7.000 U/ kg vitamin A, 700 U/kg vitamin D3, 0.4% phosphorus, 0.6 to 1.6% calcium, and 0.1 to 0.4% sodium twice a day (at 7:00 a.m. and 7:00 p.m.) based on their age and weight. Meanwhile, water and alfalfa hay were provided ad libitum.

All test animals in each group received treatment and after 15 minutes, were induced by PTZ at a dose of 80 mg/KgBW dissolved in 0.9% NaCl. Subsequently, observations on the onset, duration, and frequency of seizures were recorded at 12 hour intervals and the dose groups were compared to the positive and negative controls. Onset was calculated from the time of injection of PTZ to the start of the seizure. The duration was calculated from the start of the seizure to the end, while the frequency was the number of seizures that occurred. According to Erkec and Arihan (2015), the majority of seizures observed were tonic-clonic and the seizure score after 30 minutes of PTZ injection is defined by six phases of 0, 1, 2, 3, 4, 5, and 6, indicating no response, ear and facial twitching, myoclonic body jerks, clonic forearm seizures, generalized clonic seizures, changes to one side position, generalized clonic-tonic seizure (or death) within 30 minutes, and death, respectively.

In Vivo Anti Epilepsy Activity Test

The anti-epileptic activity of each treatment group was determined by observing the time of onset, duration, frequency, and number of deaths in test animals.

The mean of onset, duration, number of seizures, and deaths in the treatment group was compared with the control. Seizure conditions in PTZ-induced test animals were observed based on the six phases.

RESULT

In Vivo Test of Anti-Epileptic Activity

Table 1 shows the anti-epileptic activity of the treatment group based on the mean and standard deviation of time of seizure onset, duration and frequency, and the number of deaths in the test animals for each group.

Table 1: The Results of Generalized tonic-clonic Seizures in PTZ-Induced Test animals

Group	Mean ± SD			
	Seizure Onset (second)	Seizure Duration (second)	Seizure Frequency	Number of Deaths (%)
I	46.83±8.76	1015.50 ±523.39	6±1.11	3
II	1088.3±100.02	223±46.52	1±0	0
III	404.33±89.57	817.17±88.02	3±0.69	0
IV	758.67±90.15	591.17±100.93	1±0.69	0

Group I: negative control using 0.5% Na CMC suspension.

Group II: treatment with IP Sodium valproate dose of 100 mg/KgBB

Group III: treatment with Sodium valproate E tablets dose of 4.5 mg

Group IV: treatment with Mucoadhesive Buccal film sodium valproate dose of 4.5 mg

The Onset of Generalized Tonic-clonic Seizures.

Table 1 shows that there were significant differences between all the treatment groups and the negative control group. The sodium valproate administered intraperitoneally, orally, and through buccal films had anticonvulsant effects. The IP route resulted in the longest onset of activity, followed by the buccal film treatment group and then oral. Furthermore, the onset of activity is related to the effect of treatment that can prolong seizure onset.

Duration of Generalized Tonic-clonic Seizures

According to Table 1, all the treatment groups and the negative control group had significant differences. An anticonvulsant effect was observed in the treatment groups that were administered sodium valproate intraperitoneally, orally, and with buccal film. This effect can be observed in the shortening of the duration of tonic-clonic seizures. The shortest duration of activity was produced by the treatment group that received IP sodium valproate, followed by the buccal film treatment group and oral.

Frequency of Generalized Tonic-clonic Seizures

Based on Table 1, there were significant differences between all the test animals in the treatment groups and the negative control group. Sodium valproate administered intra-peritoneally, orally and through the buccal mucosa produced an anticonvulsant effect, which was observed by the reduction in the frequency of tonic-clonic seizures. Furthermore, the lowest frequency of seizures was produced by the treatment group in which sodium valproate was administered intraperitoneally, followed by the buccal film treatment group and orally.

Number of Deaths

Table 1 shows that there was a significant difference between the treatment group and the negative control group. This indicates that the treatment has the potential to act as an anticonvulsant because it can significantly reduce the number of deaths in PTZ-induced test animals. The mean, SD of onset, duration, number of seizures, and number of deaths in each group are shown in Table 1

The results of the observation showed that sodium valproate buccal film mucoadhesive preparations had a longer onset time, shorter seizure duration, and lower seizure frequency when compared to the negative control group and the experimental group of animals with sodium valproate tablets. It also produced better results when compared to the group given IP sodium valproate solution, which was able to prolong onset, shorten the duration, lower the frequency of seizures, and prevent deaths in PTZ-induced test animals.

The results of seizure onset and duration differ between treatment groups. Table 1 shows that there was a significant difference between the treatment and negative control groups at $P < 0.05$. This effect was observed from the delay in the onset of seizures. The normality value obtained is the p value (0.005) > 0.05, indicating that the data is abnormal. Using the Kruskal-Wallis test, the significance values of Onset at P (0.000) <0.05, Duration at P (0.002) < 0.05, and Frequency at P (0.000) < 0.0 were obtained. Therefore, there is a significant difference between the treatment groups, which also shows a significant difference with the negative control. Sodium valproate buccal film mucoadhesive treatment resulted in a longer onset, shorter duration, and lower seizure frequency compared to the negative control and the group receiving the slow-release (ER) tablets. Compared to the IP administration group, sodium valproate solution was also able to prolong onset,

shorten the duration and frequency of seizures, and did not cause PTZ-induced death in test animals.

Pharmacokinetic Profile Analysis

The buccal preparation of sodium valproate was administered to the test animals by opening the mouth and placing it in the buccal area. However, the test animals were previously administered ketamine anesthesia to facilitate administration and ensure that the preparation was not removed or swallowed by the test animals. Similar doses of sodium valproate (4.5 mg each) in IP form, tablets, and mucoadhesive films, were administered.

Pharmacokinetic parameters were determined individually by non-compartmental analysis using the Winsaam software program version 3.3.0. The parameters calculated include area under the concentration versus time (AUC) curve, terminal elimination half-life, pharmacokinetic parameters such as peak plasma concentration (Cmax), time to peak plasma concentration (Tmax), and area under the time curve of plasma concentration (AUC0- a).

Table 2: Pharmacokinetic Data Parameters

Parameter	IP administration	Tablet Sodium valproate	Buccal Mucoadhesive Film
Ka (hr-1)	0.0034	0.0028	0.0033
T1/2 abs (/minute)	0.67	5.12	3.46
Tmax (hr-1)	2.53	4.45	3.93
Cmax (mg/L)	3.62	4.45	4.55
AUC total	29919	195471	239061
Vd (L)	19.03	6.88	3.03
K_{el} (/minute)	0.0009	0.0017	0.0053
$t_{1/2}$ el (minute)	6.05	23.69	21.8
Cl (L/minute)	0.0894	0.0065	0.0051

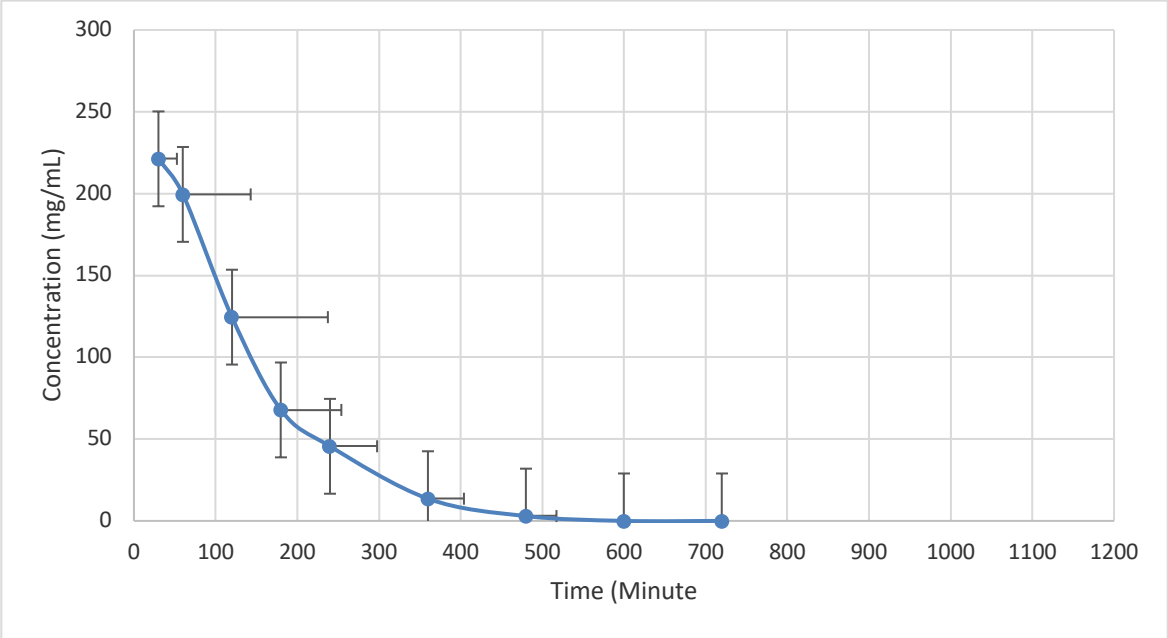

Figure 1. Plasma IP Concentration Profile of Sodium Valproate in Rats (n=6)

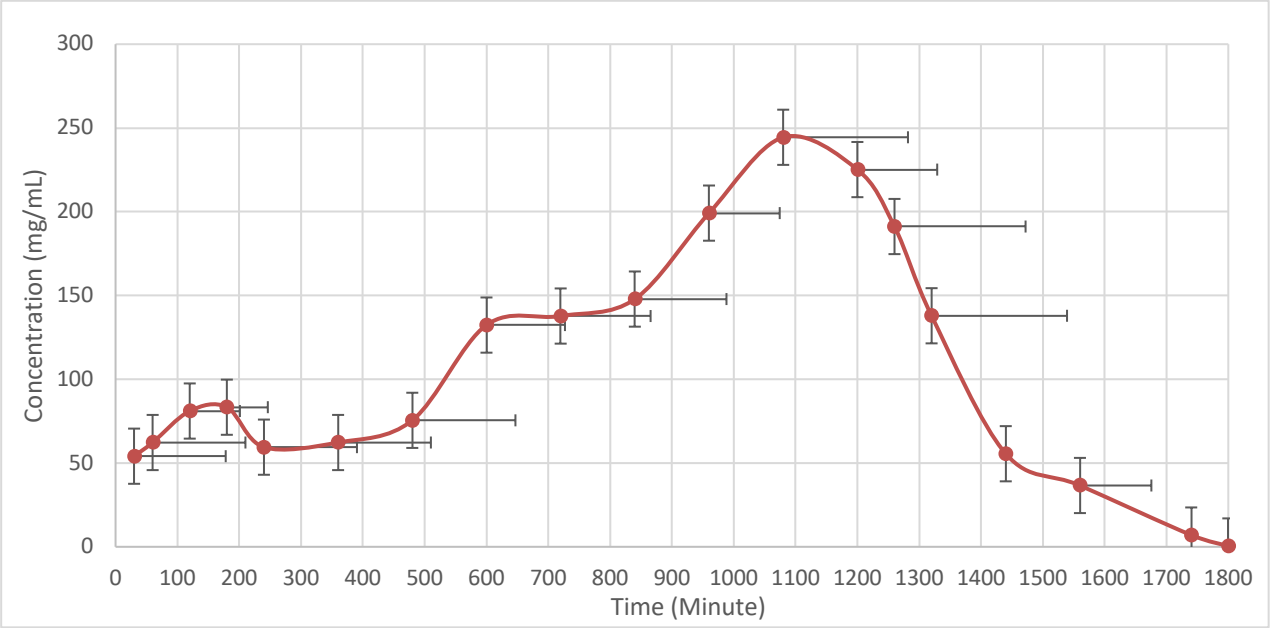

Figure 2: Plasma Concentration Profile of Sodium Valproate Tablets in Rats (n=6)

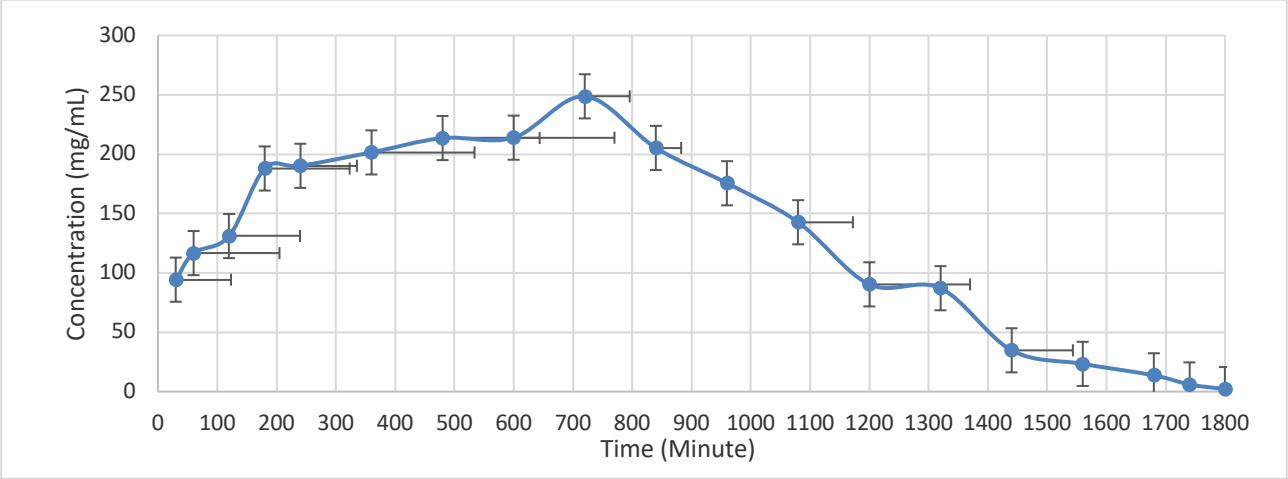

Figure 3: Plasma Concentration Profile of Sodium Valproate Buccal Mucoadhesive Films in Rats (n=6)

Based on the pharmacokinetic profile data in table 2, the absorption phase is shown as the values of Tmax, Cmax, and Kabs. Dosage form and route of administration affect the difference in the maximum level of sodium valproate in the systemic circulation. The Cmax of IP NP, sodium valproate tablets, and mucoadhesive buccal film NP was 3.62 mg/L, 4.45 mg/L, and 4.55 mg/L, respectively. While the time needed to achieve the maximum drug concentration if sorted from the shortest was 2.53, 3.93, and 4.45 minutes for IP NP, mucoadhesive buccal film NP, and tablets NP ER, respectively.

Kabs describes the speed of absorption, which is the time it takes for the drug to enter the systemic circulation from the site of absorption. The Ka abs for IP NP, mucoadhesive buccal film, and sodium valproate tablets was 0.0034, 0.0033, and 0.0028, respectively. The rate of absorption of buccal film mucoadhesive preparations was slower than IP administration but slightly greater than

sodium valproate tablet preparations. Additionally, the IP route had the lowest Tmax value of 2.53 followed by the mucoadhesive buccal film at 3.93 and sodium valproate tablets at 4.45.

The distribution phase can be determined by the volume of distribution (Vd). The greater the value of Vd, the lower the drug levels in the blood because in this phase the drugs have spread to the body's tissues. The IP NP had the highest Vd of 19.03, followed by NP ER tablets of 6.88 and mucoadhesive buccal film NP of 3.03. The volume of distribution is pharmacologically used to determine the amount of drug distribution in plasma that will remain in the body after oral or parenteral administration. It specifies the amount of drug required to be distributed and provides an estimate of the drug concentration in the blood.

The elimination phase is described by the clearance value (C1), which is the volume of blood cleared of the drug in the body. The Cl value obtained from the highest was IP NP of 0.0894 mL/minute, mucoadhesive buccal film of 0.0051 mL/minute, and NP ER tablets of 0.0065 mL/minute, indicating that every minute the amount of blood cleaned was 0.0894 mL. The AUC Total was 29919, 239061, and 195471 for IP NP, Mucoadhesive buccal film, and NP ER tablets, respectively. Furthermore, the higher the level at each time, the greater the AUC value for that time, and the longer the t1/2 of the drug, the more time it takes to reduce drug levels by half.

Figures 1, 2, and 3 compare the sodium valproate plasma levels after the IP, oral and buccal administration. When compared to IP, buccal film preparations prolong the release of sodium valproate, increase the duration of absorption, and increase the absorption of sodium valproate in the body measured by plasma concentration levels. The route of delivery also influences sodium valproate Cmax values. Furthermore, the Tmax value was found to be significantly increased after buccal administration (3.93 hours) when compared with sodium valproate tablets for the control (4.45 hours). Table 2 shows that the total AUC value for the buccal route (239061) was higher than that of the oral route, indicating increased bioavailability of sodium valproate with buccal films. A significant increase in the AUC value, as in the case of the buccal film, signifies an increase in the rate of absorption of sodium valproate from the buccal film compared to sodium valproate tablets. Therefore, the development of sodium valproate preparations in the form of mucoadhesive buccal films can affect the pharmacokinetic profile of sodium valproate and be a good alternative to provide a sustained release profile.

DISCUSSION

The results showed that the three routes of sodium valproate administration, including IP, oral and buccal, produced antiepileptic activity. The mechanism of sodium valproate antiepileptic activity is thought to be through the increase in GABA inhibition. This increases GABA-ergic neurotransmission that has been inhibited by PTZ, resulting in an anticonvulsant effect due to the inhibitory effect on the GABA GAT-1 transporter. According to a recent study, the mechanism of action of valproate is by inhibiting GABA transaminase and succinate semialdehyde dehydrogenase, which increases the GABA concentration by reducing its

degradation.[2] The administration of sodium valproate through the buccal mucosa is preferable to oral administration because it does not undergo phase one metabolism, and the permeability and supply of blood flow to the mucosal area is high enough to increase the bioavailability of sodium valproate in medical therapy.

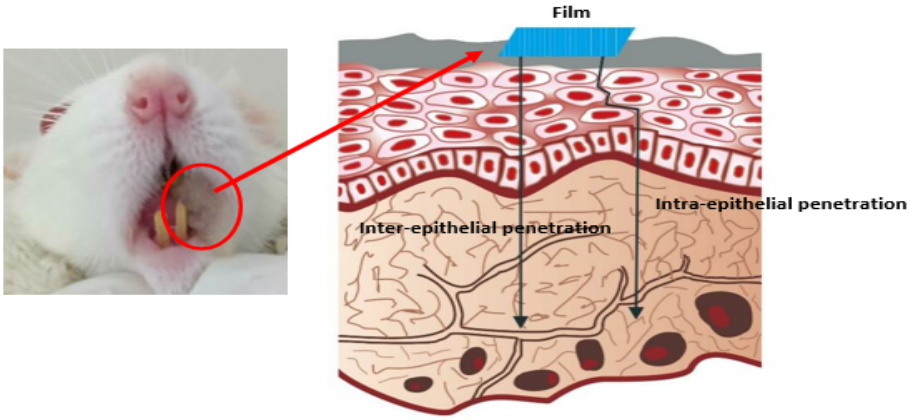

Figure 4: Illustration of the Buccal Mucoadhesive Film Release of Sodium Valproate in the Buccal Mucosa

Figure 4 shows the process of releasing the active substance to reach systemic circulation. The delayed release of the sodium valproate from the mucoadhesive buccal film is demonstrated by the pharmacokinetic profile and Tmax, which describe the time of absorption and release of the drug from the mucous membrane. Mucoadhesive buccal film preparations survive for a specific time in the mucosa, and the active ingredients trapped in the film undergo erosion and diffusion before slowly moving into the buccal mucosal solution and then undergoing a process of penetration and absorption through intra- and inter-epithelial cells and into the systemic circulation. According to Mc. Elay & Hughes 2007, there are two possible pathways for drug release through the buccal mucosa. These include the drug dissolving in the buccal fluid and entering the lymphatic and blood circulation or the drug dissolving in the buccal membrane before moving from the oral cavity due to swallowing. In this study, it is important to ensure that the buccal mucoadhesive film prepared with sodium valproate remains in the buccal area of the test animals because it is essential and is quite difficult, specifically in testing anti-epileptic activity and collecting blood plasma. The aminopeptidase activity is another factor that could explain the phenomenon of the release profile and bioavailability of drugs from macromolecular mucoadhesive buccal film preparations adsorbed on the epithelium.[6] The enzymes found in the buccal mucosa include aminopeptidase, carboxypeptidase[7] endopeptidase, leucine aminopeptidase, and cholesterol esterase. [8]

The delivery route through the buccal mucosa can also be influenced by the structure of the mucous membrane and salivary fluid, as shown in Figure 4. Therefore, the structure of the mucous membrane, which varies in various parts of the oral cavity, and reduced permeation due to obstructions influence the release of the active ingredient sodium valproate from the film preparation system. The presence of the mucosal epithelial layer and constant saliva can prevent the retention of the mucoadhesive film in one area of the oral cavity and result in a shorter contact time.[9]

CONCLUSION

Based on the results, Buccal Film Mucoadhesive preparations have

antiepileptic activity mediated by increasing GABA, thereby producing an anticonvulsant effect by elevating GABA-ergic neurotransmission inhibited by PTZ. Therefore, prolonged onset, shorter seizure duration, and lower seizure frequency were obtained compared to the negative control and the extended-release sodium valproate tablet group. Preparations in the form of buccal mucoadhesive films can affect the pharmacokinetic profile with different absorption parameters of Ka, Tmax, Cmax, Vd, T1/2 el, and Kel compared to commercial preparations.

ACKNOWLEDGMENTS

The authors are grateful to the Dean of the Faculty of Pharmacy, Padjadjaran University and the Chief of the National College of Health Sciences for their support. The authors also express gratitude to PT. Otto Pharmaceutical laboratories for the donations of Sodium Valproate used for experiments.

REFERENCES

1. Falco-Walter J. Epilepsy—Definition, Classification, Pathophysiology, and Epidemiology. Semin Neurol. 2020;40(06):617-623.
2. Rahman M, Nguyen H. Valproic Acid. In: StatPearls. StatPearls Publishing; 2022. Accessed December 13, 2022. http://www.ncbi.nlm.nih.gov/books/NBK559112/
3. Perucca E. Pharmacological and Therapeutic Properties of Valproate. Mol Diag Ther. 2002;16(10):695-714.
4. Virmani T, Singh C, Sorout G, Virmani R, Gupta J. Buccal Drug Delivery system- Approach to Improve the Bioavailability. Published online 2015:7.
5. Ergul Erkec O. Pentylenetetrazol Kindling Epilepsy Model. Epilepsi. Published online 2015.
6. Morales JO, McConville JT. Manufacture and characterization of mucoadhesive buccal films. European Journal of Pharmaceutics and Biopharmaceutics. 2011;77(2):187-199.
7. Obradovic T, Hidalgo IJ. In Vitro Models for Investigations of Buccal Drug Permeation and Metabolism. In: Ehrhardt C, Kim KJ, eds. Drug Absorption Studies. Vol VII. Biotechnology: Pharmaceutical Aspects. Springer US; 2008:167-181.
8. Yamahara H, Lee VHL. Drug metabolism in the oral cavity. Advanced Drug Delivery Reviews. 1993;12(1-2):25-39.
9. Bhati R, Nagrajan RK. A Detailed Review on Oral Mucosal Drug Delivery System. International Journal of Pharmaceutical Sciences and Research. 2012;3(1):23.