Effect of Irbesartan-Poloxamer-188 Solid Dispersion on Intercellular Cell Adhesion Molecule-1 and Interleukin-8 on Hypertension Rats

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Abstract

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BACKGROUND: Based on the Biopharmaceutics Classification System (BCS) system, irbesartan is a drug that belongs to the class II BCS group which has limitations in terms of dissolution rates with low bioavailability of 26% -60%. These limitations to bioavailability can be overcome by solid dispersion with hydrophilic matrices such as Poloxamer. Irbesartan is an angiotensin receptor blocker. At present, it is widely used in dealing with hypertension due to endothelial dysfunction.

AIM: This study aims to determine endothelial function blood markers can be examined, such as adhesion molecules (ICAM-1) and IL-8 pro-inflammatory cytokines.

MATERIAL AND METHODS: Research on the effects of irbesartan-poloxamer-188 solid dispersion on ICAM-1 and IL-8 in hypertensive rats has been carried out. The formation of solid dispersion through dissolution method while induction of hypertension using 2.5% NaCl and prednisone 1.5 mg/Kg BB orally in 3 treatment groups, irbesartan dose was 13.5 mg/kg. The parameters observed were serum ICAM-1 and IL-8 levels.

RESULTS: The result showed that the solid dispersion of irbesartan-poloxamer-188 could reduce ICAM-1 and IL-8 levels in hypertensive rats which differed significantly from the positive control group ($p < 0.05$).

CONCLUSION: This study concluded that the solid dispersion of irbesartan-poloxamer-188 effects and decreases ICAM-1 levels in the serum of hypertensive rats. Solid dispersion of irbesartan-poloxamer-188 can influence and reduce IL-8 in the serum of hypertensive rats.

Introduction

Based on the Biopharmaceutics Classification System (BCS) system, irbesartan is a drug that belongs to the class II BCS group, which has limitations in terms of dissolution rate [1], [2]. Availability of irbesartan is reported to be 26% [3] and 60% [4]. This limitation of bioavailability has been overcome by the solid dispersion of irbesartan made with dextrose water-soluble matrix, the method of making solid dispersion is done by grinding and smelting method [5], formulation and evaluation of irbesartan liquid-solid tablets to improve irbesartan dissolution and bioavailability [2].

Another study of solid dispersion technology using super disintegrant sodium starch glycolate, crospovidone, croscarmellose sodium and microcrystalline cellulose [3]. The latest study was comparing the dissolution rate of 2 methods of making irbesartan tablets namely wet granulation technique and sublimation technique [6].

To improve the effectiveness of treating hypertension using irbesartan, it is necessary to look for new polymers. Poloxamer-188 is often considered a functional excipient because it is an important component in the formulation. Regarding the amphiphilic structure possessed by this surfactant, it is widely used in the industry. In the study of irbesartan, solid dispersion with various comparisons of poloxamer-188 the best dissolution rate was obtained at a ratio of 2:1 [7].

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In the inflammatory process, the endothelial surface will express adhesion molecules such as vascular cell adhesion molecule-1 (VCAM), intercellular cell adhesion molecule-1 (ICAM) and interleukin-8 (IL-8) [4]. On the other hand, the effect of dissolution rate and modification of the crystal properties of irbesartan on endothelial cells such as intercellular cell adhesion molecule-1 (ICAM) and interleukin (IL-8) has not been reported.

Material and Methods

Research Materials

Irbesartan (Dr Reddys), poloxamer-188 (Merck), ethanol 96%, prednisone, NaCl, NaCMC and distilled water, ELISA kits for ICAM-1 and IL-8 (USCN).

Instruments

Vacuum ovens, desiccators, digital analytic scales (Denver Instruments), UV-Vis spectrophotometers (UV-1700 PharmaSpec), ELISA reader.

Animal Experiments

White mice were weighing between 200-300 grams many as 24 (*Rattus norvegicus*) Wistar strain (Laboratory of Pharmacology), Faculty of Pharmacy, Andalas University, Padang.

Acclimatised animals

For the next 7 days were grouped into 4 groups. Three groups of experimental animals were given induction with 2.5% NaCl and prednisone 1.5 mg/kg body weight as much as 2 mL orally for 2 (two) weeks; then the experimental animals were given a test preparation orally at a dose of 13.5 mg/kg for 1 (one) week. Each group consisted of 6 rats and treated as follows: Group I as a negative control, was given standard food and drink, group II as a positive control, standard food and drinks were given and given induction, group III as a test group was given standard food and drinks and inducers and irbesartan non-dispersion dose of 13.5 mg/kg, group IV as a test group given standard food and drink and induction and solid dispersion of irbesartan the dose was equivalent to 13.5 mg/kg. Blood is taken from the eye vein by 1.5 ml at certain minutes. Then the separation between serum and blood objects was carried out, the serum was stored in a refrigerator and the storage cabinet for a sample temperature of -40°C for further analysis.

Test for Irbesartan Solid Dispersion Activity against ICAM-1 levels

All reagents are prepared, use well, according to the number of wells plate used and labelled. Well plate for standard solutions determined, blanks and samples, made 7 wells for standards and 1 for blanks, added 100 µL of each standard solution, blanks, and samples into appropriate wells, covered with incubation plate sealers for 2 hours at 37°C. The solution of each well was removed and not washed and added 100 µL of the working solution to detect the reagent in each well and incubated for 1 hour at 37°C after being covered with plate sealer.

The supernatant was removed and washed with 350 µL of wash buffer, with 3 x inverted plates. Next 100 µL of reagent B working solution was added to each well; the plate sealer was closed, incubated for 30 minutes at 37°C. The washing process was repeated up to 5 times, added 90 µL of the substrate solution to each well, covered with a new plate sealer and incubated for 15 minutes at 37°C and protected by the light the solution would turn blue. Fifty µL stop solution is added to each well, so the solution turns yellow. Then the microplate reader is traced with an ELISA at a wavelength of 450 nm to determine its optical density value. The results of ICAM-1 level determination, in each rat serum at a specified time compared to each experimental group.

Examination of Levels of IL-8

All reagents are prepared and use wells according to the number of wells used and labelled. Well for standard solutions, blanks and samples are determined, 7 wells for the standard (1000, 500, 250, 125, 62.5 and 31.25 pg/mL) are made and 1 for blanks, 100 µL of each standard solution is added, blank and the sample into the right well, covered with a sealer plate and incubated for 1.5 hours at 37°C. The solution of each well was removed and not washed and added 100 µL of the working solution to detect reagent (biotin-labelled antibody) into each well and incubated for 1 hour at 37°C after being covered with plate sealer. The supernatant was removed and washed with 350 µL wash buffer, doing 3x inverted plates. Then added 100 µL of the working solution (SABC) into each well, covered with a plate sealer and incubated for 30 minutes at 37°C. The washing process was repeated up to 5 times, added 90 µL TMB substrate solution to each well, covered with plate sealer new and incubated for 15 minutes at 37°C and protected by the light the solution would turn blue. 50 µL stop solution is added to each well, so the solution turns yellow. The microplate reader is then run by using ELISA at a wavelength of 450 nm to determine its optical density value. The results of IL-8 level determination, in each rat serum at a specified time compared to each experimental group.

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Statistical analysis

The results of the non-dispersion evaluation of irbesartan and solid dispersion of irbesartan-poloxamer-188 on serum ICAM-1 and IL-8 levels were analysed using statistics IBM SPSS version 19. Normal distribution tests are carried out using the Saphiro Wilk test. If the value of sig. > 0.05, then the data is normally distributed. For further analysis, one-way parametric (ANOVA) is conducted. To determine the significance of the treatment, the Bonferroni Post Hoc test was carried out.

Research Ethics Requirements

The approval of the Ethics Commission of the Faculty of Medicine, Unand Padang with ethical clearance No. 395/KEP/FK/2017.

Results

Effect of giving irbesartan solid dispersion on ICAM-1 levels

The measurement results of the average ICAM-1 levels of each negative control group rat were: 5.97 ± 2.32 ng/mL, the positive control group were: 16.68 ± 1.30 ng/mL, the group of mice given the preparation irbesartan non-dispersion is 13.11 ± 2.48 ng/mL and the group of rats given irbesartan-poloxamer -188 solid dispersion preparation is 9.54 ± 1.04 ng/mL.

Table 1: Results of ICAM-1 examination in rat serum

NO	I (ng/mL)	II (ng/mL)	III (ng/mL)	IV (ng/mL)
1	7.36	17.01	12.99	8.63
2	7.62	18.42	9.82	10.79
3	3.56	17.36	13.43	10.13
4	7.62	16.61	16.83	9.65
5	2.46	17.19	14.50	7.97
6	7.22	14.45	11.06	10.046
X ± SD	5.97 ± 2.32	16.68 ± 1.3	13.11 ± 2.48	9.54 ± 1.04

These results showed that after induction, there was an increase in ICAM-1 levels in the positive control group whereas after irbesartan administration both non-dispersion and solid dispersion ICAM-1 levels decreased, as shown in Table 1.
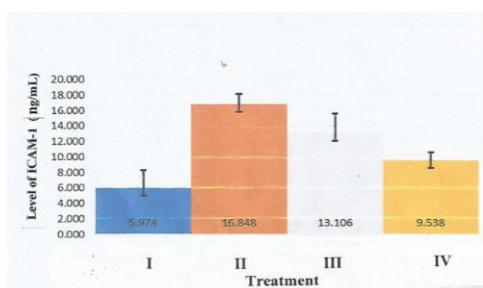

Figure 1: Levels of ICAM-1 in treatment animal group; I) Negative control; II) Positive control; III) Non-dispersion of irbesartan; IV) Solid dispersion of irbesartan

Effect of treatment on IL-8 levels

The administration of inducible substances in the positive control group caused an increase in IL-8 levels in the blood.

Table 2: Results of IL-8 examination in rat serum

NO	I (pg/mL)	II (pg/mL)	III (pg/mL)	IV (pg/mL)
1	43.88	52.29	49.47	41.56
2	38.19	50.04	46.65	41.01
3	39.89	50.04	50.60	44.96
4	39.32	50.60	42.71	45.53
5	40.45	53.98	46.09	45.53
6	43.27	52.86	42.14	47.22
X±SD	40.83 ± 2.26	51.63 ± 1.65	46.27 ± 3.43	44.30 ± 2.46

Decreased level of IL-8 was found in the treatment group after induction and administration of irbesartan non-dispersion and solid dispersion of irbesartan (Table 2 and Figure 2).
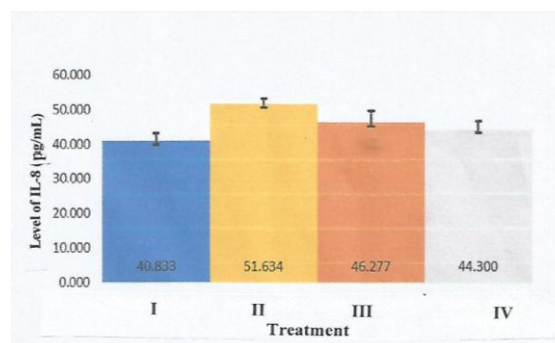

Figure 2: Levels of IL-8 in treatment animal group; I) Negative control; II) Positive control; III) Non-dispersion of irbesartan; IV) Solid dispersion of irbesartan

Discussion

ICAM-1 level

The results showed that after induction, there was an increase in ICAM-1 levels compared to negative controls of 16.85 and 5.47 ng/ml. Hypertension is an inflammatory process that involves the migration and accumulation of cells from innate and adaptive immune responses into the interstitium of blood vessels by releasing cytokines and increasing oxidative stress. Oxidative stress which plays a role in the pathogenesis of endothelial dysfunction in hypertension is from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, integrin kinase 1 as a mechanism for the reduction in vascular superoxide production due to hypertension [8], [9].

The inflammatory process that occurs with changes in blood vessels that arise quickly and in the short term. This response occurs because the mediators of bradykinin and histamine produced by local mast cells cause vasodilation and increased permeability. This permeability phase occurs within a few minutes. After 30-60 minutes, there is a marginalisation of neutrophils which group along the

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endothelium in the injury area, followed by the migration of blood fertilisers. The migration process occurs through the process of margination, adhesion, rolling (grinding), stop rolling and diapedesis [10].

At the stage of the process of marginalisation, the activated macrophages will produce mediators of proinflammatory cytokines, namely IL-1 and TNF-α. Furthermore, TNF-α will induce vascular endothelial cells to express adhesion molecules, namely selectin, integrin, immunoglobulin superfamily (ICAM-VCAM, mucin-like molecule, and CD44. There are three types of ICAM namely ICAM-1, ICAM-2, and ICAM-3. ICAM-1 and ICAM-2 are expressed by endothelial cells and also APC, where these bonds allow lymphocytes to migrate through the walls of blood vessels and there is a weak involvement between the molecules so that the leukocytes attach to endothelium [10].

In the rolling stage, the weak attachment between leukocytes and endothelium will be stronger, so that the strength of the bloodstream cannot release this bond. The attachment between leukocytes and endothelium becomes increasingly strong because of activation by chemotactic factors such as leukotriene B4, platelet-activating factor and IL-8 by increasing the affinity of leukocyte adhesion molecules for endothelial adhesion molecules The higher the blood pressure, the inflammatory response that occurs will also increase to protect the body from cell injury and a sustained inflammatory response will increase various proinflammatory cytokines, ICAM molecules and chemotactic factors IL-8 [10], [11].

The mean ICAM-1 levels from the group given irbesartan non-dispersion and solid dispersion of irbesartan-poloxamer-188, decreased by 13.106 ng/mL and 10.2014 ng/mL when compared to positive controls (16.847 ng/ml), but the results of the decline ICAM-1 levels did not reach the same value as the negative control (5.474 ng/ml). This is because animals that have experienced endothelial dysfunction so that recovery takes time.

Irbesartan, as one of the angiotensin blocker receptor drugs in the form of non-dispersion and solid dispersion, can inhibit the angiotensin II response binding to specific angiotensin receptors. Finally, the administration of this drug can improve endothelial function, increase vasodilator mediators and increase NO bioavailability and reduce the concentration of CRP (C-reactive Protein) and inflammatory markers such as IL-6, IL-1, ICAM and other factors like chemokine IL-8 [12].

The results of one-way analysis of variance analysis (ANOVA) on examination of ICAM-1 levels in experimental animals showed a significant difference between the groups given irbesartan to the positive control group. Based on the results of the Post Hoc Bonferroni statistic, there was a significant effect between negative controls with positive controls and non-dispersion groups and no significant effect on the solid dispersion group.

The results of the research that have been conducted show that the solid dispersion with poloxamer-188 carriers has an effect of decreasing the average ICAM-1 serum level with ICAM-1 levels lower than irbesartan non-dispersion. This is related to changes in the physicochemical properties of irbesartan can increase the rate of dissolution of the drug, with an increase in the dissolution rate of a drug and absorption of the drug so that the expected effect is also achieved [13].

Level of IL-8

The mean value of IL-8 levels from the group given irbesartan non-dispersion preparation and solid dispersion of irbesartan-poloxamer-188 decreased by 46.28 pg/mL and 42.80 pg/mL when compared to positive controls (51.63 pg/ml), but the result of a decrease in IL-8 levels has not reached the same value as the negative control (40.83 pg/ml).

The results of the research that have been carried out show that the solid dispersion with poloxamer-188 carriers has an effect of decreasing the serum levels of IL-8 with IL-8 levels which are lower than non-dispersible irbesartan. This is because there has been a change in the physicochemical properties of irbesartan so that solubility, dissolution, and bioavailability have increased [13]. The powder X-ray analysis showed the decreasing in peak intensity at $2\theta = 12.34°$ from 6345.9 to 2915.3, which indicates the formation of a crystal lattice that has a degree of symmetry. Decreasing the intensity of the interference peak shows changes in the degree of crystallinity so that it will increase its solubility [7].

The results of one-way analysis of variance analysis (ANOVA) on IL-8 levels in experimental mice showed a significant difference between the groups given the preparation to the positive control group. Based on the results of the Post Hoc Bonferroni statistic with positive and non-dispersion controls, the significant influence between negative controls with positive control and non-dispersion irbesartan was significant ($p < 0.05$) and did not significantly influence the solid dispersion group ($p > 0.05$).

In this study, the result showed that the solid dispersion of irbesartan could affect the physicochemical properties of irbesartan, decreasing the level of ICAM-1 and IL-8 levels in the serum of hypertensive mice. Decreased levels of ICAM-1 and IL-8 showed a decrease in the level of inflammation in endothelial cells so that the permeability of endothelial cells be better. This is proven by previous studies of a decrease in mice blood pressure and an increase in serum NO levels [7].

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Acknowledgements

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