- Ref: 156-6
- Indonesia

THE DESIGN OF A LIVER-SELECTIVE FORM OF INTERLEUKIN-10: A new strategy for the treatment of liver fibrosis

Summary of the thesis

Heni Rachmawati

inflammatory processes during acute glomerulonephritis induced by anti-Thy 1 after 24 h. A possible anti-inflammatory mechanism underlying this acute effect of IL-10 may occur via inhibition of glomerular ICAM-1 expression, resulting in reduced macrophage recruitment as reflected by reduced CD14 staining (**chapter 4**). The reduced MMP-13 and proteinuria after IL-10 treatment indicates that this action of IL-10 is beneficial to preserve glomerular integrity. However, despite decrease in inflammatory parameters at the protein levels and the glomeruloprotective effects in IL-10-treated rats, an effect of IL-10 on the expression of many genes was not found in the time frame studied in this experiment.

In a subsequent study we examined the effects of IL-10 in advanced glomerulonephritis in rats (**chapter 5**). A high amount of IL-10 accumulated within one minute in the kidney and remained present in this tissue up to 60 minutes (gammacamera results), yet this accumulation probably reflects uptake in tubular cells. So, in view of the short plasma half-life, we assessed the effect of a daily administration of IL-10 on a chronic process like glomerulosclerosis. We administered IL-10 from day 4 to day 6 and sacrificed the animals at day 7. In this time frame, initiation of disease has taken place, and acute inflammation is already strongly diminished but the process of glomerulosclerosis is still rapidly ongoing. As presented in **chapter 5**, we found potent effects of IL-10 treatment in this model. IL-10 interfered with almost all of the parameters that eventually affect matrix deposition. However, the inhibition of the fibrotic process evidently did not lead to attenuation of the glomerular damage; proteinuria was not reduced by IL-10 treatment. IL-10 treatment did also not affect the mRNA levels for various examined genes in the time frame defined in this experiment.

In view of the results presented in **chapter 4** and **chapter 5**, we can conclude that IL-10 exerts acute and more chronic effects *in vivo* within the kidney. The extensive renal clearance of IL-10, however limits a clinical application of this cytokine for the treatment of chronic liver diseases like liver fibrosis. In order to enhance the effectivity of this cytokine for the treatment of liver fibrosis, this cytokine has to be delivered to this organ to overcome the normal low uptake. We designed a modified form of IL-10 with a specific ligand for the M6P/IGF-II receptor. This receptor is highly expressed on activated HSC. The coupling reaction of mannose 6-phosphate (M6P) to IL-10 was performed with

micrograms of protein. Characterization of the product is therefore a major challenge since this limited amount of protein does not allow the use of classical methods. Determination of mannose 6-phosphate groups in the conjugate was unsuccessful with classical sugar assays and with classical phosphate assays. Determination of a successful coupling with a mass spectrophotometry also failed due to the limited amount of the coupling products. Western blotting method was therefore used to characterize the conjugate because of its sensitivity and its specificity. Using this method, two bands of M6PIL-10 representing a monomeric and a dimeric conjugate were detected. Also native IL-10 was characterized by a monomeric and a dimeric form, of which only the latter is bioactive.[24] In addition to immunodetection, we also performed bioassays to assess the activity of the conjugate *in vitro*. In this *in vitro* system, we used RAW cells which constitutively express IL-10 receptors but have no M6P/IGF-II receptors and primary isolated HSC which expresses both IL-10 and M6P/IGF-II receptors. The inhibitory effect of M6PIL-10 on the release of TNF-α by LPS-stimulated RAW cells was almost equivalent to that of IL-10, indicating that pharmacological activity of IL-10 on an important inflammatory parameter is preserved in the modified-IL-10. In addition, the inhibitory effect of the conjugate on the collagen deposition and on the induction of MMP-13/TIMP-1 mRNA ratio in HSC (two crucial parameters of fibrogenesis) was also almost equivalent to that of IL-10. The effectivity of the conjugate on HSC reveals a binding capacity of the conjugate to the IL-10 receptor.

The improvement of drug concentration at the desired site of action is a major goal in designing targeted drug delivery systems. We describe in **chapter 6** respectively **chapter 7**, the preferential hepatic homing of M6PIL-10 in normal rats and in rats with liver fibrosis (BDL). By coupling M6P to IL-10, the biodistribution profile of IL-10 clearly shifted from the kidney to the liver. A study on the acute effects of M6PIL-10 in nephritic rats induced by anti-Thy 1 IgG, yielded an absence effect of the conjugate (data not shown), in contrast to unmodified IL-10. This result confirms the biodistribution data, that is, M6PIL-10 concentration is too low within the kidney to exhibit a biological effect in this model of kidney disease.

In this study, we could not identify a cell-selective delivery of the conjugate using standard double immunostaining methods. This is due to the fact that high concentrations of IL-10 can not be achieved *in vivo*. We therefore performed an *in vivo* study to identify the target receptor for M6PIL-10. Radiolabeled IL-10 and M6PIL-10 was administered to rats together with several agonists for the putative target receptors for M6PIL-10. Competitive hepatic uptake between M6PIL-10 and various receptor blocking proteins indicated that three different receptors in the liver i.e. the M6P/IGF-II, the scavenger and most likely the IL-10 receptors contributed to the hepatic uptake of our conjugate (**chapter 6**). The blockade of hepatic accumulation of [^{125}I]M6PIL-10 with either $M6P_{24}$HSA or sucHSA clearly reflects this involvement of M6P/IGF-II and scavenger receptors. In contrast, preadministration of either $M6P_{24}$HSA or sucHSA did not influence the hepatic uptake of [^{125}I]IL-10. Although $M6P_{24}$HSA and sucHSA inhibited M6PIL-10 uptake, there was no additive effect when both proteins were combined. Since IL-10 receptors are present in the liver (**chapter 3**) and since our experiments with RAW cells indicate that M6PIL-10 also binds to this receptor (**chapter 6**), IL-10 receptor is most likely also involved in the hepatic disposition of this conjugate. Blocking the IL-10 receptor with an excess amount of IL-10 is needed to prove this hypothesis, but these high sustained concentrations can not be achieved *in vivo* due to the rapid clearance of IL-10.

As three different receptors are responsible for the hepatic uptake of the conjugate, a complex interaction between M6PIL-10 and the target cell is anticipated. To illustrate this complexity, a proposed model is depicted in chapter 6 (fig.8). The accessory receptors depicted in this figure are the M6P/IGF-II receptor and the scavenger receptor.

The pharmacological activities of M6PIL-10 that we demonstrated in this thesis indicate that M6PIL-10 binds to IL-10 receptors. Even an improvement of the therapeutic effectiveness of the conjugate in BDL-1 rats, as compared to unmodified IL-10 was noted on some parameters (**chapter 7**). This can be explained by an enhanced concentration at the target cell (chapter 7, fig.1). Since IL-10 receptor density is low, even after its upregulation during disease[24,25], a sustained increased concentration of IL-10 around the target cell is important for the biological activity. In the *in vivo* studies, liver fibrosis in rats was induced by

ligation of the bile duct. This procedure leads to inflammatory responses in the liver and proliferation of bile ducts.[26-28] These processes are subsequently followed by proliferation of HSC and portal fibroblasts and a large production of matrix components by this cell type. Positive stainings for α-SMA, desmin/GFAP and type III collagen around the portal areas reveal this fibrogenesis process, whereas induction of iNOS, IL-10 receptor, and DAB staining in the portal area and around the necrotic area reveals the hepatic inflammatory activity. Induction of the mRNA levels for TNF-α, MMP-13, ICAM-1, procollagen type I, TGFβ-1, α-SMA, and TIMP-1 compared to normal liver also clearly reflect the fibrogenesis in the first week after ligation. Treatment with either IL-10 or M6PIL-10 was given for 3 consecutive days from day 4 to day 6 after induction of the disease. In this time frame, both IL-10 and M6PIL-10 suppressed liver inflammation and fibrosis reflected a reduction in several parameters examined. IL-10 and M6PIL-10 significantly reduced DAB positive staining in the portal area and M6PIL-10 showed slight superior effect than IL-10 on some of the parameters. In particular, the superior effect of M6PIL-10 on the reduction of collagen deposition as compared to IL-10 (chapter 7, fig.5) might indicate that a liver-selective delivery of IL-10 improves the therapeutic efficiency of this cytokine. However, additional dose-response studies are required to address this issue. No significant differences on the mRNA levels for various genes between untreated and treated groups were seen. The data for IL-10 and M6PIL-10 were similar in this respect.

Conclusion

The studies presented in this thesis describe all activities in designing a targeting system for a therapeutic cytokine, from the documentation of the potential effects of this cytokine in various diseases, the synthesis of a cell-selective form of IL-10, the pharmacokinetic and organ distribution profile including receptor interactions *in vivo*, to the testing of the targeted cytokine in diseased animals. Cytokine-based research is revolutionizing the treatment of several diseases including liver fibrosis. However, the classical problem with the clinical use of cytokines is that the administration of these proteins must be by injection, either intravenously or subcutaneously. In addition, cytokines often have short plasma half-lives, due to rapid renal excretion and proteolytic degradation in plasma, whereas their activity on cells is usually most optimal

after long exposure times. As a consequence, they have to be administered frequently, which makes them very expensive as a drug. The clinical use of many cytokines is also limited because of their pleiotropism. Because their receptors are expressed in a number of cells and tissues, systemic application of the cytokines can easily result in undesired effects. The strategy to target the cytokine to its receptor ensures a rapid delivery of the cytokine to the site of action, while avoiding non-target sites and thereby may eliminate undesired effects mostly observed during long-term cytokine therapies. This can be achieved by coupling a receptor-selective ligand to the cytokine. Using mannose 6-phosphate (M6P), we selectively delivered a potent antifibrotic cytokine, IL-10, to the liver. The conjugate is pharmacologically active *in vitro* and in an animal model of liver fibrosis *in vivo*.

A short-term daily single dose of M6PIL-10 during liver fibrosis induced by bile duct ligation revealed potent effects of the conjugate in controlling the inflammatory and the fibrotic processes. IL-10 and its modified form influenced several parameters associated with the fibrotic process, and eventually affected the deposition of fibrous tissue in the liver. We even found some beneficial effects of M6PIL-10 in controlling these processes as compared to unmodified IL-10. This result indicates that an efficient delivery of IL-10 to the target cell in a diseased organ is possible after its modification with a HSC-selective ligand.

Future perspectives

Many types of endogenous proteins, such as cytokines, are produced and released to act locally in the body, thus exhibiting the desired activity among their multiple functions. However, the exogenous administration of such proteins into the systemic circulation may result in serious side effects due to their low targeting efficiencies to the site of action. This could be one of the stumbling blocks that hinder their clinical application. A selective delivery of the proteins to the site of action is therefore necessary to overcome such problems. As described in this thesis, IL-10 exerts potent antifibrotic effects both *in vitro* and in a model of liver fibrosis in rats. The hepatic targeting system for IL-10 using a HSC-selective ligand, M6P, shows great potential for future therapeutic applications for the treatment of liver fibrosis. Since up till now, no antifibrotic drugs are approved

due to safety problems, a potent antifibrotic effect of a liver-selective form of IL-10 during liver fibrosis offers great new opportunities.

In addition to this liver-selective form of IL-10, another interesting strategy to achieve a local sustained high level of IL-10 during hepatic fibrogenesis is now ongoing in animal experimentations.[29-31] IL-10 gene transfer has recently been proposed to have potential therapeutic applications since local gene expression can yield high local protein level for a prolonged period of time (up to months). This sustained IL-10 expression within the liver may also be a promising strategy for the treatment of chronic liver diseases like liver fibrosis. In particular liver fibrosis, where systemic effects of drugs often oppose the therapeutic effects within the liver, may benefit from such a cell-selective delivery of drugs.

Heni Rachmawati

CURRICULUM VITAE

Name	: Heni Rachmawati
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Development of nanoparticulate-based drug targeting

Nanotechnology-inspired approaches to particle design and formulation, an improved understanding of (patho)physiological processes and biological barriers to drug targeting, as well as the lack of new chemical entities in the 'pipeline', are causing large pharmaceutical companies problems in bringing new drug compounds to the market. This indicates that there is a bright future for targeted nanoparticles as pharmaceuticals. Targeting systems can target a drug to the intended site of action in the body, thus enhancing its therapeutic efficacy (site-specific delivery), and/or direct a drug away from those body sites that are particularly sensitive to the toxic action of it (site-avoidance delivery).

The development of effective, safe, and innovative drug targeting systems, is a complicated multi-step process. There is an increasing need to select and/or identify appropriate matrix materials, surface coatings, and targeting ligands with advanced properties. Therapeutic agents (small molecules, but also macromolecules like proteins and nucleic acids) to be loaded into nanocarriers vary widely in their physicochemical properties and it remains a challenge to balance the nanoscale dimensions of the particulate with the types and amounts of drugs that are clinically required. Proper structural and physicochemical characterization is required to guarantee reproducible effects *in vivo*. In addition to the development of nanomedicines for systemic targeting, the development of nanomedicines that release the drug locally in diseased parts of the gastrointestinal tract (*e.g.* collonic targeting for colon cancer therapy), after oral administration, will also yield distinct improvements compared with existing, non-specific, drug delivery methods.

A. Publication in international journals

1. **Heni Rachmawati,** Leonie Beljaars, Catharina R-Smit, Werner I.Hagens, Dirk F. Meijer, Klaas Poelstra, **2004,** Pharmacokinetic and Biodistribution profile of recombinant human interleukin-10 in rats with extensive liver fibrosis, *Pharmaceutical research, 2004, 21:2072-2078.*

2. **Heni Rachmawati,** Leonie Beljaars, Catharina R-Smit, Hester I.Bakker, Dirk F.Meijer, Klaas Poelstra, A study of the effects of interleukin-10 in anti-Thy 1-

induced **glomerulonephritis** in rats, *Nephrology, 10 (Supll.), 2005.*

3. **Heni Rachmawati,** Leonie Beljaars, Catharina R-Smit, Hester I.Bakker, Dirk F.Meijer, Klaas Poelstra, A study of the effects of interleukin-10 in anti-Thy 1-induced **glomerusclerosis** in rats, *Nephrology, 10 (Supll.), 2005.*

4. **Heni Rachmawati,** Leonie Beljaars, Catharina R-Smit, Hester I.Bakker, Dirk F.Meijer, Klaas Poelstra, IL-10 modified with mannose 6-phosphate yields a liver-selective cytokine, Drug Metab.Dispos., 35(5):814-21, 2007.

5. Sukmadjaja Asyarie, Faizatun, **Heni Rachmawati,** *In vitro and in vivo evaluation of* solid dispersion system of gliclazide:PEG 6000, PDA Journal of Pharmaceutical Science and Technology, Vol 61(5) p.400-410, 2007.

B. Publication in National Journal

1. Heni Rachmawati, I Ketut Adnyana, Sukmadjaja Asyarie, "Uji efek hipoglikemik sistem dispersi padat gliklazid:PEG 6000 pada tikus yang diinduksi aloksan, Artocarpus, Vol6, 85-90, 2006.
2. Sukmadjaja Asyarie, Pricilia, **Heni Rachmawati,** Formula tablet kaptopril lepas lambat dengan matriks pautan silang alginate, Majalah Farmasi Indonesia, 18(1),2007, 34-39.
3. Heni Rachmawati, Betty Wert, Catharina Smith, DKF Meijer, Klaas Poelstra, Development of a mannose 6-phosphate-modified human serum albumin construct: A cell-specific carrier to hepatic stellate cells, Journal Acta Pharmaceutica Indonesia, Vol 32(2), June 2007.

C. Publication in International Conferences

1. **Heni Rachmawati**, Leonie Beljaars, Catharina R-Smit, Werner I.Hagens, Dirk F. Meijer, Klaas Poelstra, Pharmacokinetic and Biodistribution profile of recombinant human interleukin-10 in rats with extensive liver fibrosis, *European Conference on Drug Delivery and Pharmaceutical Technology*, Seville, Spain, May, 2004.

2. **Heni Rachmawati**, Leonie Beljaars, Catharina R-Smit, Hester I.Bakker, Dirk F. Meijer, Klaas Poelstra, Chemically modified interleukin-10 with a liver-selective ligand: a new strategy for the treatment of liver fibrosis with a therapeutic cytokine, *Keystone symposia: Cytokine, Disease, and Therapeutic Intervention*, Santa Fe-New Mexico, USA, February, 2005.

3. **Heni Rachmawati**, Leonie Beljaars, Catharina R-Smit, Hester I.Bakker, Dirk F. Meijer, Klaas Poelstra, Chemically Modification of interleukin-10 with mannose 6-phosphate yields a liver-selective cytokine with antifibrosis activities in rats: *40th Annual Meeting of The European Association for The Study of The Liver*, Paris, France, April, 2005.

4. **Heni Rachmawati,** Leonie Beljaars, Catharina R-Smit, Hester I.Bakker, Dirk F.Meijer, Klaas Poelstra, A study of the effects of interleukin-10 in anti-Thy 1-induced **glomerulonephritis** in rats, *3rd World Congress of Nephrology,* Singapure, June, 2005

5. **Heni Rachmawati,** Leonie Beljaars, Catharina R-Smit, Hester I.Bakker, Dirk F.Meijer, Klaas Poelstra, A study of the effects of interleukin-10 in anti-Thy 1-induced **glomerusclerosis** in rats, *3rd World Congress of Nephrology,* Singapure, June, 2005.

6. **Heni Rachmawati,** Leonie Beljaars, Catharina R-Smit, Hester I.Bakker, Dirk F.Meijer, Klaas Poelstra, A study of the effects of interleukin-10 in anti-Thy 1-induced **glomerulonephritis** in rats, *Renal Disease in Minority Populations and Developing Nations* , Singapure, July, 2005.

7. **Heni Rachmawati,** Leonie Beljaars, Catharina R-Smit, Hester I.Bakker, Dirk F.Meijer, Klaas Poelstra, A study of the effects of interleukin-10 in anti-Thy 1-induced **glomerusclerosis** in rats, *Renal Disease in Minority Populations and Developing Nations* , Singapure, July, 2005.

8. **Heni Rachmawati,** Ferry Damanhuri, Debbie S.Retnoningrum, Pegylation of Recombinant Mutein Streptokinase resulted from overproduction in E.coli BL 21 and study on the fibrinolytic activity in vitro, oral presentation, Federation of Asian Pharmaceutical Association, Singapore, November 2008.

9. Debbie Retnoningrum, Ratih A.Ningrum, Yohannes S.Kurniawan, **Heni Rachmawati,** Construction, cloning and overproduction of recombinant human interferon alpha 2b resulted from gene synthesize using TBIO method, Federation of Asian Pharmaceutical Association, Singapore, November 2008.

D. Publication in International Proceeding

1. Meta Santika, **Heni Rachmawati**, Yeyet Cahyati, Sustained Release Tablet Formulation of Diltiazem Hydrochloride Using Hydrophillic Matrix Hydroxypropyl Methylcellulose, ICMS Proceeding, 2007.
2. Faizatun, **Heni Rachmawati**, Sukmadjaja, IN VITRO AND IN VIVO EVALUATION OF GLICLAZIDE ORAL SOLID DOSAGE FORMS IN RATS, ICMNS Proceeding, 2007.
3. Lita Vidianty, **Heni Rachmawati**, Yeyet Cahyati, Formulation of Turmeric (Curcuma domestica Val.) Ethanol Extract into Effervescent Tablet using PEG 4000 as a solubilizing enhancer, ICMNS Proceeding, 2007.
4. Hestiary Ratih, **Heni Rachmawati**, Sundani Nurono, PAPAIN MICROENCAPSULATION BY EMULSIFICATION SOLVENT EVAPORATION TECHNIQUE USING HPMCP HP-55, ICMNS Proceeding, 2007.

5. Lia, Meliani, **Heni Rachmawati**, Joseph I.Sigit, The method development of piroxicam-gentamicin induced renal failure in rat model, ICMNS Proceeding, 2007.
6. **Heni Rachmawati,** Faizatun, Yatina Sari, Sukmadjaja Asyarie, Physical Identification of Binary System of Gliclazide-Hydrophilic Polymers Using X-Ray Diffraction , Proceeding, International Conference on Neutron and X-ray Scaterring, Indonesia, 2007.
7. **Heni Rachmawati,** M.Saifullah Amin, Development of colonic tablet containing ibuprofen using in situ alginate crosslinking, Proceeding International Conference on Mathematics and Natural Sciences, Indonesia, October 2008.
8. **Heni Rachmawati,** Dita Herawati, Sasanti Tarini, Folic acid encapsulation in stearic acid-based solid lipid nanoparticle, Proceeding International Conference on Mathematics and Natural Sciences, Indonesia, October 2008
9. **Heni Rachmawati,** Endah Pratiwi, Sukmadjaja Asyarie, Formulation of effervescent pellet containing green tea extract, Proceeding International Conference on Mathematics and Natural Sciences, Indonesia, October 2008.
10. **Heni Rachmawati,** Fitria Mufida, Jessie S.Pamudji, Formulation and characterization of matrix-floating tablet of riboflavin, Proceeding International Conference on Mathematics and Natural Sciences, Indonesia, October 2008.
11. Ni Made Hegard Sukmawati, Joseph I.Sigit, **Heni Rachmawati**, Effect of Corn Silk (Maydis stigma) Extract on Histology and Gene Expression of Gentamicin and Piroxicam Induced Renal Rat Model, Proceeding International Conference on Mathematics and Natural Sciences, Indonesia, October 2008.
12. I Ketut Adnyana, Neng Any Tadjudin, **Heni Rachmawati**, Hepatoprotective Activity of Saponin Fraction of Oyong Seeds (Luffa acutangula (L.) Roxb) on Liver Fibrocytic Rat-induced with CCl4, Proceeding International Conference on Mathematics and Natural Sciences, Indonesia, October 2008.
13. Debbie S.Retnoningrum, Aniek Widayanti, **Heni Rachmawati,** Overproduction and purification of streptokinase K59Q-K386Q, and its stability assay to plasmin cleavage using Western blot, Proceeding International Conference on Mathematics and Natural Sciences, Indonesia, October 2008.

E. Invited speaker

1. **Heni Rachmawati,** Leonie Beljaars, Catharina R-Smit, Hester I.Bakker, Dirk F.Meijer, Klaas Poelstra, Synthesis and characterization of mannose 6-phosphate-modified human serum albumin (M6P-HSA), a cell specific carrier to hepatic stellate cells, Pharmaceutical research Seminar Nasional, Bandung, Indonesia, September 2002.
2. **Heni Rachmawati,** Research methodology in pharmaceutics, Competitive grant A1, Department of pharmacy, University of Islamic Indonesia, Yogyakarta, Maret 2006.

3. **Heni Rachmawati**, Development strategy of protein formulation, in short course: Recent development and future perspective of recombinant protein in industry", School of Pharmacy ITB, 2005.
4. **Heni Rachmawati**, Recombinant protein in drug delivery for cancer therapy, in short course: Recent development and future perspective of recombinant protein in industry", School of Pharmacy ITB, 2005.
5. **Heni Rachmawati**, Recombinant protein for the treatment of infectious disease: HCV and HIV, in short course: Recent development and future perspective of recombinant protein in industry", School of Pharmacy ITB, 2005.
6. **Heni Rachmawati,** Nanomedicine in drug delivery system and its prospective development in Indonesia, Institut Teknologi Bandung, Indonesia, 2005.
7. **Heni Rachmawati,** The incidence of hepatitis virus infection in Indonesia and the strategy to combat the disease, International Symposium on Characterization and Management of Viral Disease in The Developing World, Faisalabad – Pakistan, November 2006.
8. **Heni Rachmawati,** The prospect of recombinant proteins in therapy, The 5th Asia Pacific Pharmaceutical Symposium, Bandung, 2006.
9. **Heni Rachmawati,** External triggers in drug delivery system, The 9th conference in instrumentation and control, Institut Technology Bandung, Indonesia, February 2007.
10. **Heni Rachmawati**, Future perspective of nanotechnological application in pharmaceuticals, Nanotechnology State of the Art in Health Care and Pharmaceuticals, Jakarta-Indonesia, August, 2007.
11. **Heni Rachmawati,** Molecular pathogenesis of hepatitis virus infection and cellular targeting of drug for the treatment, The 1st international symposium on molecular pathogeneses: "Recent advantages on molecular pathogenesis and application to pharmaceutical product development, Bandung-Indonesia, January 2008.
12. **Heni Rachmawati,** Recombinant Cytokines and their pharmaceutical uses, Workshop: Synthetic genes and their ususe for various applications, Bandung-Indonesia, January 2008.
13. **Heni Rachmawati,** Nanoencapsulation of protein for oral delivery purpose, International workshop on nanomedicine, Islamabad-Pakistan, Maret 2008.

F. Research grants

- Islamic Development Bank, Jeddah, Saudi Arabia, 2001-2004.
- Ubbo Emmius Scholarship, University of Groningen, The Netherlands, 2005.
- Competitive research grant, Bandung Institute of Technology, 2006 (PI)
  "*In vitro and in vivo evaluation of* solid dispersion system of gliclazide:PEG

6000"

- Research Grant ITB 2006 (Team Member)
  "The method development of piroxicam-gentamicin induced renal failure in rat model."
- PT Sorini, Jakarta (research collaboration between ITB and PT Sorini, 2005) (PI)
  "Formulation of paracetamol syrup with improved water solubility of paracetamol"
- PT Kalbe Farma (research collaboration between ITB-PT Kalbe Farma) (PI)
  "Stability study of capsule containing of garlic and curcumin"
- PT Bayer Indonesia (research collaboration between ITB and PT Bayer, 2006) (Team member)
  "Reformulation of syrup containing multivitamins and minerals"
- Research Grant from ITB, 2007 (PI)
  "Utilization of a natural polymer for delivering of macromolecule for oral administration"
- Research grant ITB 2007 (Team member)
  "Increase of streptokinase stability against plasmin degradation via pegylation technique."
- Applied research grant, Ministry of Research and Technology, Indonesia, 2007 (PI)
  The construction and cloning of synthetic interferon alpha 2b coding sequence in E.Coli"
- Applied research grant, Ministry of Research and Technology, Indonesia, 2008 (PI):
  "Over-expression, purification, and characterization of interferon alpha 2b produced from cloning of synthetic interferon alpha 2b coding sequence"
- Applied research grant, Ministry of Research and Technology, Indonesia, 2008 (Team member)
  "Development of release system of Bone Morphogenetic Protein-2 localized in multilayer implant to accelerate the regeneration of bone"
- Research Grant ITB, 2008 (Team member)
  "Study on the effect of Maidis extract in piroxicam and gentamycin-induced renal failure in rat"
- Research Grant ITB, 2008 (Team Member)
  "Study on the effect of tropical plant extract in CCl4-induced chronic liver fibrosis in rat".
- PHKA3 School of Pharmacy ITB (PI)
  "Colonic tablet of ibuprofen: formulation approach and pharmacokinetic study"

G. Related experience in research and education

1. Short course on *Working with Radioactive compounds, Sponsored by Groningen University Institute for Drugs Exploration, Groningen, The Netherlands, October 2001.*
2. Short course on *Bioinformatics, Sponsored by Groningen University Institute for Drugs Exploration Groningen, The Netherlands, November 2002.*
3. Short course on Development of methods for detection of cytokines, *Sponsored by Islamic Development Bank (Saudi Arabia), London (England), 2002.*
4. Short course on Controlled Drug Release, *Sponsored by Islamic Development Bank, Noordwijk aan Zee, The Netherlands, April 2004.*
5. Short course on Project Management for Scientific Research, *Sponsored by Groningen University Institute for Drugs Exploration, Groningen, The Netherlands, May 2004.*
6. Short course on The Animal Science, *Sponsored by Groningen University Institute for Drugs Exploration, Groningen, The Netherlands, May 2004.*
7. Short course on Proteomics – Functional Genomics, *Sponsored by Groningen University Institute for Drugs Exploration and GBB Ruhs-University Bochum Germany, Bochum, Germany, June 2004.*
8. Short course on Safe Microbiological Techniques, *Sponsored by Groningen University Institute for Drugs Exploration, Groningen, The Netherlands, September 2004.*
9. Short course on Good Manufacturing Practice, *Sponsored by Groningen University Institute for Drugs Exploration, Groningen, The Netherlands, September 2004.*
10. Short course on Drug Delivery and Drug Targeting, *Sponsored by Groningen University Institute for Drugs Exploration, Groningen, The Netherlands, May 2004.*
11. Short course on Techniques in Molecular Biology, *Sponsored by Groningen University Institute for Drugs Exploration, Groningen, The Netherlands, January 2005 2004.*
12. Short course on *Good Laboratory Practice and Good Clinical Practice, Sponsored by Groningen University Institute for Drugs Exploration, Groningen, The Netherlands, may 2005.*

G. Teaching experiences

Undergraduate program

1. Pharmaceutical Solid Dosage Form (2000-present)
2. Unit processes in pharmacy (2000-present)

3. Law and ethics in Pharmacy (2005-present)
4. Pharmaceutical compounding (2008)
5. Pharmaceutical Biotechnology (2005-present, partly)

Professional (apotheker)

Law and Ethics in Pharmacy (2005-present)

Postgraduate program

1. Physicochemistry of pharmaceutical solid (2000-2001)
2. Unit operation in Pharmacy (2005-present)
3. Technology of DNA recombinant (2005-present, partly)

H. Miscellaneous

11.1. Published books

1. Heni Rachmawati, Klaas Poelstra, Leonie Beljaars, *Cytokine and Modified-Cytokine as therapeutic agents: Present State and Future Perspectives*, in Book: Recent Research Development in Immunology, Publisher: Research Signpost, Kerala, India, 2004, 6:191-214, ISBN: 81-7736-206-2.
2. Heni Rachmawati, *The Design of a liver-selective form of interleukin-10: a new strategy for the treatment of liver fibrosis, dissertation, Publisher: University of Groningen, Groningen, The Netherlands, June 2005, ISBN: 90-367-2272-1.*

11.2. International Awards and travel grants

1. Young investigator award, Department of Health and Human Services, National Institutes of Health USA, National Institute of Allergy and Infectious Diseases, February 2005.
2. EASL Young Investigator's Bursaries (full Roche Unrestricted Education Grant), The European Association for the Study of the Liver, April 2005.
3. International society of Nephrology (ISN), June, 2005.
4. Travel grant from COMSTECH (committee on Scientific and Technological Cooperation), Pakistan, 2006
5. Travel grant from Ministry of Cultural and Education, Indonesia, 2006.
6. Travel grant from PT Biofarma, Indonesia, 2006.
7. Travel grant from COMSTECH-OIC, Pakistan, 2008.
8. Ristek-Kalbe Young Scientist Award, Indonesia 2008.

Committee

1. Organizing Committee of International Conference on Mathematics and Natural Sciences 2006 and 2008, Bandung-Indonesia, 2006.

2. Organizing committee of International Seminar on Pharmaceutics, Bandung-Indonesia, 2007.
3. Organizing committee of The 1st International Symposium on Molecular Pathogenesis, Bandung-Indonesia, 2008.

I. MEMBERSHIP

1. Indonesian Pharmacist Association
2. Member of Indonesian Society for Nanotechnology

J. REVIEWER

Reviewed research papers and research proposals:

1. PDA Journal of Pharmaceutical Science and Technology (International Journal)
2. Drug Development and Industrial Pharmacy (International Journal)
3. LPPM ITB (national)