

# Perspectives of pharmacological intervention promoting liver regeneration

## Perspektywy farmakologicznej interwencji w promowaniu regeneracji wątroby

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### Summary

Effective drug therapy promoting liver regeneration is a challenging goal in pharmacotherapy of liver diseases. Several plant phytochemicals recommended in traditional medicine from over hundred plants have been investigated for its use in various liver disorders. Regeneration of injured liver depends on a proliferative potential of mature hepatocytes as well as different subsets of intrahepatic and extrahepatic stem/progenitor cells. In clinical trials a stem cell therapy resulted in a limited improvement of liver functions. Animal tests have demonstrated the involvement of bone marrow-derived stem/progenitor cells in liver regeneration. For this reason, the pharmacological activation of endogenous stem cells and pharmacological control of macrophage phenotypic polarization could be an effective method of mobilizing progenitor cells to injured liver.

**Key words:** liver regeneration, stem cells therapy, macrophage reprogramming, pharmacotherapy of liver diseases

### Abbreviations:

ALF: acute liver failure, DILI: drug-induced liver injury, HBV: hepatitis B virus, HCV: hepatitis C virus, NALFD: non-alcoholic fatty liver disease, PH: partial hepatectomy, PHLF: post-hepatectomy liver failure.

### Limited hepatoprotective potential of drugs

Hepatoprotective drugs are very limited [1, 2]. Effective drugs that stimulate hepatic function, offer complete protection to the organ, or help to regenerate hepatic cells, is a challenging question in modern pharmacology. Non-alcoholic fatty liver disease (NALFD), considered as an independent risk factor in pre-diabetes, is the most common liver disease. Post-hepatectomy liver failure (PHLF), remaining as life-threatening complications of hepatectomy, takes place in up to 10% of cases [3]. Progressive fibrosis after chronic liver injury can be effectively stopped or reversed only after removing the causative agent. Corticoster-

oids use does not improve overall survival in drug-induced indeterminate- or autoimmune acute liver failure (ALF). Until now there are no approved treatments for alcoholic liver disease. The risk of drug-induced liver injury (DILI) increases with different preexisting liver diseases. Drugs described as hepatotoxic were classified in five categories in a newly developed open access LiverTox (<http://livertox.nih.gov>). About 150 medicinal plant phytochemicals recommended in traditional medicine from over hundred plants have been investigated for its use in various liver disorders [4]. Hepatoprotective phytochemicals and synthetic or semi-synthetic pharmaceuticals can help in liver injury, depending on their bioavailability, relative low toxicity and anti-inflammatory potential.

### Regenerative potential of liver

Maintaining liver-to-body-weight ratio, regardless the type of injury, is required for homeostasis [5]. Proliferative potential of quiescent mature hepatocytes is stimulated by a diverse range of stimuli, capable of inducing approximately 95% of the hepatic cells to enter a replicative state G1/S and subsequent increase in the liver mass. The regenerative process stops after coordinated intervention of inhibitory cytokines, to return

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liver cells to quiescent state, once an appropriate liver-to-body weight ratio is achieved. Liver regeneration does not necessarily depend exclusively on a proliferative potential of mature hepatocytes. Different subsets of intrahepatic and extrahepatic stem/progenitor cells are believed to take part in liver regeneration [6]. Human clinical tests have shown that hematopoietic stem cells were mobilized into the circulation of patients after liver resection in amounts proportionate to the extent of surgery [7]. Thanks to animal tests, different types of liver recovery are currently recognized, based on different types of proliferating cells: mature, normally quiescent hepatocytes are activated to proliferate and regenerate the organ following injuries due to toxins, drugs, acute viral diseases or partial hepatectomy (PH). Reserve progenitor cells, also normally quiescent in the liver, are activated by severe liver injury [8]. Bone-marrow-derived cells were found in the liver of healthy animals [6], as well as in regenerating liver after PH [9]. Thus, liver regeneration may not only depend on proliferation of intact hepatocytes and local stem cells, but also can be related to migration of stem cells and macrophages from bone marrow [10, 11]. Thus, in animal models, depending on the cause and severity of injury, liver regeneration was divided into three distinct restoring levels: hepatocyte dominant regeneration, participation of liver stem/progenitor cells, and involvement of extrahepatic stem/progenitor cells [6].

### Pharmacological mobilization and recruitment of stem/progenitor cells

The impact of liver stem cell therapy in several clinical trials is currently interpreted as weak, unable to modify the clinical course of severe liver diseases [12]. However, depending on liver disease/injury and clinical trial protocol, a stem cell therapy resulted in a limited improvement of liver functions [13]. Patients showed a more important liver macrophagic expansion as compared to standard treatment, after transarterial administration of bone marrow-derived stem cells. The treatment, however, did not increase proliferative hepatocyte number. Patients with a significant improvement of liver function were characterized at baseline by a significant number of proliferating hepatocytes, proliferative progenitor cells and higher macrophage infiltration, as compared to non-improvers [12].

Numerous animal tests have demonstrated the involvement of bone marrow-derived stem cells in liver regeneration. For this reason, the pharmacological activation of endogenous stem cells could be a simple and effective method of presenting stem cells to injured liver. In rat model, pharmacological mobilization of bone marrow cells promoting liver regeneration has been recently evidenced after extensive liver resection [10]. Engraftment of CD133<sup>+</sup> stem cells in the remnant liver and increased proliferation of hepatic double-stained OV6/Ki67 oval cells was obtained in animals pretreated with combination of two drugs: AMD3100 (Plerixafor or Mozobil) and low dose of FK506 immunosuppres-

sant. Thus, synergistic action of these two drugs resulted in mobilization and recruitment of bone marrow stem cells [10].

### Pharmacological control of macrophage reprogramming and phenotypic polarization

Macrophages can respond to various stimuli in a spectrum of activation, including tissue remodeling by macrophages [14]. Several lines of evidence suggest that macrophages and dendritic cells are able to abort the pathological immune response by M1 → M2 phenotypic polarization and producing anti-inflammatory cytokines [4]. Hepatic Kupffer cells display markers of M1-like macrophages or M2-like macrophages depending on the signals from their environment. A potential role for macrophage polarization has been recently examined in promoting pancreatic recovery and  $\beta$ -cell proliferation. Macrophages of the M2 phenotype prevented the destruction of  $\beta$ -cells, pancreatic islets and the development of diabetic nephropathy in experimental diabetes [15]. In rat model of alloxan-induced diabetes, phthalhydrazide treatment markedly decreased interleukin 6 (IL-6) and cortisone concentration, increased the number of pancreatic islets/mm<sup>2</sup>, which resulted in a 3-fold increase in the number of insulin-producing cells [16]. Phthalhydrazide is sold for decades in pharmacies of Russian Federation as the immunomodulating and antioxidant pharmaceutical [17, 18]. A limited phthalhydrazide monotherapy for patients suffering from prolonged hepatitis B (HBV) and hepatitis C (HCV) infections (11 months–12 years) resulted in decreased concentration of serum IL-1 and TNF- $\alpha$ , and decreased enzymatic markers of liver damage [19]. Normal bilirubin level and less pronounced clinical signs of the disease such as diminished fatigue, appetites improvement, decreased skin itching and other signs of health improvement were noted among these patients showing good tolerability and the absence of adverse effects related to the therapy. Thus, phthalhydrazide therapy decreased signs of viral hepatic disease and normalized liver functions. Conversely, chronic activation of monocytes and macrophages correlated with liver damage in HCV infection [20].

In mouse model of PH liver injury, phthalhydrazide treatment of hepatectomized animals improved macrophage recruitment to liver and increased the number of proliferating/binuclear K67<sup>+</sup> hepatocytes [11]. Other signs of improved liver regenerative process, including decreased number of focal necrosis, anisocytosis, anisonucleosis, increased blood albumin- and total blood protein content, were evident after phthalhydrazide treatment, when compared to control hepatectomy. [11]. Generally, PH triggered activation of monocytopenesis in the bone marrow, resulting in approximately 2-fold increase in the number of bone marrow monocytes/macrophages, whereas phthalhydrazide treatment of animals further promoted the hepatectomy-induced infiltration of macrophages into liver stroma.

## Perspectives and conclusions

Regeneration of injured liver is important therapeutic target in liver diseases. Stem cell transplantation can be possibly an alternative to liver transplantation being much safer and less invasive. Adipose-derived stem cells, with their ability to differentiate into hepatic lineage and increase in the number of hepatocytes, are considered suitable candidates for human liver regeneration [21]. Liver cells repopulation can be achieved by engraftment of transplanted cells, but most transplanted cells are rapidly cleared from liver sinusoids by proinflammatory cytokines/ chemokines/ receptors. Biological drug Etanercept, an anti-TNF-alpha-biopharmaceutical, decreased transplanted cell clearance and caused several-fold acceleration of liver repopulation [22]. Macrophage reprogramming and pharmacological control of phenotypic polarization of macrophage cells can possibly prevent the onset of inflammatory response in injured organ and reduce production of macrophage proinflammatory cytokines [4]. Pharmacotherapy of bone marrow cells engraftment into the remnant liver and increased proliferation of hepatic cells [10] can be possibly achieved in humans in the near future.

## Piśmiennictwo/References

[1] Ortiz-Lopez, C., Lomonaco, R., Orsak, B., Finch, J., Chang, Z., Kochunov, V. G., ... & Cusi, K. (2012). Prevalence of prediabetes and diabetes and metabolic profile of patients with nonalcoholic fatty liver disease (NAFLD). *Diabetes care*, 35(4), 873–878.

[2] Karkhanis, J., Verna, E. C., Chang, M. S., Stravitz, R. T., Schilsky, M., Lee, W. M., & Brown, R. S. (2014). Steroid use in acute liver failure. *Hepatology*, 59(2), 612–621.

[3] Kwon, Y. J., Lee, K. G., & Choi, D. (2015). Clinical implications of advances in liver regeneration. *Clinical and molecular hepatology*, 21(1), 7–13.

[4] Danilova, I.G., Kalota, H., et al. (2018). Pharmaceuticals in liver failure and regeneration: From hepatotoxicity to hepatoprotective potential of drugs. *Almanach* (Pol) 2018, in press.

[5] Michalopoulos, G. K. (2017). Hepatostat: liver regeneration and normal liver tissue maintenance. *Hepatology*, 65(4), 1384–1392.

[6] Liu, W. H., Ren, L. N., Wang, T., Navarro-Alvarez, N., & Tang, L. J. (2016). The involving roles of intrahepatic and extrahepatic stem/progenitor cells (SPCs) to liver regeneration. *International journal of biological sciences*, 12(8), 954.

[7] Lehwald, N., Duhme, C., Wildner, M., Kuhn, S., Fürst, G., Forbes, S. J., ... & Schulte am Esch, J. (2014). HGF and SDF-1-mediated mobilization of CD133+ BMSC for hepatic regeneration following extensive liver resection. *Liver International*, 34(1), 89–101.

[8] Riehle, K. J., Dan, Y. Y., Campbell, J. S., & Fausto, N. (2011). New concepts in liver regeneration. *Journal of gastroenterology and hepatology*, 26(s1), 203–212.

[9] Tsolaki, E., & Yannaki, E. (2015). Stem cell-based regenerative opportunities for the liver: state of the art and beyond. *World journal of gastroenterology*, 21(43), 12334.

[10] Zhai, R., Wang, Y., Qi, L., Williams, G. M., Gao, B., Song, G., ... & Sun, Z. (2018). Pharmacological Mobilization of Endogenous Bone Marrow Stem Cells Promotes Liver Regeneration after Extensive Liver Resection in Rats. *Scientific reports*, 8(1), 3587.

[11] Danilova, I.G., Yushkov, B.G., et al. (2018). Migration of macrophages and bone marrow stem cells to regenerating liver promoted by sodium phthalhydrazide in mice. *Biomed. Pharmacother.* BIOPHA\_2018\_1254, submitted.

[12] Lanthier, N., Lin-Marq, N., Rubbia-Brandt, L., Clément, S., Goossens, N., & Spahr, L. (2017). Autologous bone marrow-derived cell transplantation in decompensated alcoholic liver disease: what is the impact on liver histology and gene expression patterns?. *Stem cell research & therapy*, 8(1), 88.

[13] Tayyeb, A., Azam, F., Nisar, R., Nawaz, R., Qaisar, U., & Ali, G. (2017). Regenerative Medicine in Liver Cirrhosis: Promises and Pitfalls. In *Liver Cirrhosis-Update and Current Challenges*. InTech, 236–256.

[14] Koyama, Y., & Brenner, D. A. (2017). Liver inflammation and fibrosis. *The Journal of clinical investigation*, 127(1), 55–64.

[15] Zheng, D., Wang, Y., Cao, Q., Lee, V. W., Zheng, G., Sun, Y., ... & Harris, D. C. (2011). Transfused macrophages ameliorate pancreatic and renal injury in murine diabetes mellitus. *Nephron Experimental Nephrology*, 118(4), e87–e99.

[16] Danilova, I. G., Bulavintceva, T. S., Gette, I. F., Medvedeva, S. Y., Emelyanov, V. V., & Abidov, M. T. (2017). Partial recovery from alloxan-induced diabetes by sodium phthalhydrazide in rats. *Biomedicine & Pharmacotherapy*, 95, 103–110.

[17] Abidov, M.T. (2016). N-heterocyclic pharmaceuticals for humans. *Almanach* (Pol), 11, 74–80.

[18] Abidov, M.T. (2018). New N-heterocyclic pharmaceuticals for humans. Part II. Pharmacological and biochemical characterization of hydrazide derivatives. *Almanach* (Pol), 12, 73–80.

[19] Nagoyev, B.C., Abidov, M.T., et al. (2005). In: [Chronic viral hepatitis: Immunopathogenic, clinical-diagnostic and therapeutic aspects] (Russian), Guripp Adigea Maikop.

[20] Li, H., & Tu, Z. (2017). The Role of Monocytes/Macrophages in HBV and HCV Infection. In *Biology of Myelomonocytic Cells*. InTech, Chapter 4.

[21] Yang, D., Wang, Z. Q., Deng, J. Q., Liao, J. Y., Wang, X., Xie, J., ... & Lü, M. H. (2015). Adipose-derived stem cells: A candidate for liver regeneration. *Journal of digestive diseases*, 16(9), 489–498.

[22] Viswanathan, P., Kapoor, S., Kumaran, V., Joseph, B., & Gupta, S. (2014). Etanercept blocks inflammatory responses orchestrated by TNF- $\alpha$  to promote transplanted cell engraftment and proliferation in rat liver. *Hepatology*, 60(4), 1378–1388.

**Streszczenie**

Wyzwaniem dla współczesnej farmakoterapii są skuteczne leki wspomagające regenerację wątroby. Wiele substancji z ponad stu roślin zalecanych w tradycyjnej medycynie przebadano w celu ich zastosowania w chorobach wątroby. W procesie regeneracji wątroby biorą udział subpopulacje wewnątrz-wątrobowych i poza-wątrobowych komórek macierzystych/progenitorowych oraz dochodzi do proliferacji dojrzałych hepatocytów. Dane kliniczne wskazują na ograniczoną skuteczność terapii komórek macierzystych w polepszeniu funkcji wątroby. W badaniach modeli zwierzęcych regeneracji wątroby wykazano udział komórek macierzystych/ progenitorowych, pochodzących ze szpiku kostnego. Z tego powodu, rozważa się możliwość wprowadzenia farmakologicznej aktywacji endogennych komórek macierzystych oraz farmakologiczną kontrolę fenotypowej polaryzacji makrofagów, jako skutecznej metody mobilizacji komórek progenitorowych dla uszkodzonej wątroby.

**Słowa kluczowe:** regeneracja wątroby, terapia komórek macierzystych, przeprogramowanie makrofagów, farmakoterapia chorób wątroby

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