



Relationship Between Hepatotoxicity That Induced by CCL4 and Regucalcin Protein Marker

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ABSTRACT

This review deals with the world's most important problem, which is hepatotoxicity. As a result of the distinctive location of the liver and its effect on all the substances entering the body and manufactured from it, the wastes resulting from metabolism, as well as its functional nature in the equation and removal of toxins. Therefore, the liver is one of the most influential and first organs in the human body because of the major functions it shows. Therefore, most of the research targets the liver through a number of chemicals, including carbon tetrachloride. Carbon tetrachloride is widely used, and its work depends on destroying hepatocytes through a group of chemical reactions with a group of chemical compounds for the liver such as fats and proteins, thus producing harmful substances such as free radicals, whose damage depends on the concentration and period of exposure to carbon tetrachloride. In order to determine the extent of the influence of the liver, there must be evidence. Our choice of Regucalcin was because it is closely related to several physiological functions, including: its role in maintaining the level of calcium on a regular basis in addition to its anti-programmed effects of cell death by inhibiting factors that break down the DNA strand by inhibiting the action of a group of enzymes. And other factors that destroy cells. Thus, the presence of Regucalcin inside and out of cells is evidence of the extent of damage and damage to hepatocytes and their dissolution. Therefore, Regucalcin can be considered a criterion for assessing the extent and degree of damage to the liver.

Keywords: Regucalcin, carbon tetrachloride, Hepatotoxicity.

Article Information

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HEPATOTOXICITY:

The liver disorders are one of the world problems. Despite its frequent occurrence, high morbidity and high mortality, its medical management are currently inadequate, so far not yet any therapy has successfully prevented the progression of the hepatic disease, even though newly developed drugs have been used to treat chronic liver disorders, these drugs have often side effects. Therefore, it is essential to find suitable herbal drugs that could replace the chemical ones (Jawad, Murtadha M. Homady, Merza H. and Aldujaili, Arshad N., 2018).

Liver injury due to chemicals (or) infectious agents may lead to progressive liver fibrosis and ultimately cirrhosis and liver failure

(Szymonik-Lesiuk et al., 2003). However, no effective treatment that delays disease progression and complications has yet been found. Recent studies suggest that traditional herbs and micronutrients such as carotenoids and selenium may be useful for this purpose (Murugesan et al.,2009).

CARBON TETRACHLORIDE

Carbon tetrachloride CCL4 is widely used for experimental induction of liver damage (Miyazaki et al., 2009). The principle role of carbon tetrachloride (CCL4) in inducing hepatic damage in lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals (Tirkey et al.,2005; Ohata et al., 2008).

Carbon tetrachloride has been widely used as a model of toxicity-induced chronic liver injury, progressing to fibrosis and cirrhosis (Khan et al., 2009; Kim et al., 2010a). Even though the model does not mimic the injury pattern in human HCV infection, it provides a useful tool for understanding the mechanism underlying the HCV-mediated fibrogenesis, where a similar pattern of inflammatory changes is seen (Sreelatha et al., 2009). Furthermore, the histopathological changes (fatty degeneration, centrilobular necrosis, fibrosis, cirrhosis and even cancer), alterations in liver function, severe malnutrition and the disturbances in plasma levels of branched-chain amino acids observed in cirrhotic rats are similar to those observed in human cirrhosis (Xiao-hui et al., 2007; Adewale et al., 2014). The model, in addition, provides an alternative to predict a true anti-fibrotic drug effect (Prakash et al., 2008).

REGUCALCIN PROTEIN MARKER: STRUCTURE OF REGUCALCIN:

Regucalcin (RGN) was discovered in 1978 as a Ca^{2+} -binding protein that does not contain the EF-hand motif of Ca^{2+} binding domain (Yamaguchi., 2011a). Because of its relationship to ageing and its molecular mass of 30 kDa, this peptide was also designated senescence marker protein-30 (SMP30) (Lai et al., 2011).

The regucalcin gene (*rgn*) is localised on the X chromosome (Yamaguchi., 2011b), and its gene is identified in over 15 species consisting of the regucalcin family, and the gene species are highly conserved in vertebrate and invertebrate species (Yamaguchi., 2013a). The expressions of regucalcin mRNA and protein are regulated through various transcription factors which are involved in cell signal transduction with stimulation of hormone and cytokine (Yamaguchi, 2013b).

Regucalcin is evolutionarily conserved only in higher animals and this gene is not found in yeast (Arai et al., 2009). The amino acid sequence of mouse RGN showed 94% similarity to rat RGN and 89% to human RGN. In fact, the entire primary structure of RGN is conserved among humans and rodents, suggesting that its complete structure is required for the physiological function of RGN

(Sato et al., 2012). The primary structure of RGN protein did not show the known Ca^{2+} -binding domain, such as an EF-hand motif, and it may represent a calcium binding protein of a novel type (Amano et al., 2010).

The Ca^{2+} -binding constant was found to be $4.19 \times 10^5 \text{ M}^{-1}$ by equilibrium dialysis, and there appear to be six or seven high-affinity binding sites for Ca^{2+} per molecule of protein. The hydrophathy profile of RGN showed that there was a hydrophobic sequence in both N-terminal and C-terminal regions of the RGN molecules. However, the protein showed a hydrophilic character as molecule (Iwama et al., 2011). The regucalcin molecule contains aspartic acid (24 residues) and glutamic acid (16 residues) which were suggested to be related with Ca^{2+} -binding (Nakagawa and Yamaguchi, 2006). Recently, the crystal structure of the human RGN has been determined by X-ray diffraction and the protein has a 6-bladed β -propeller fold, and it contains a single metal ion Figure (1). Also, both Zn^{2+} and Ca^{2+} -bind to the same metal-binding site in an identical manner. This is interesting, as normally the coordination of Zn^{2+} is quite distinct from that of Ca^{2+} in enzymes (Yamaguchi, 2005).

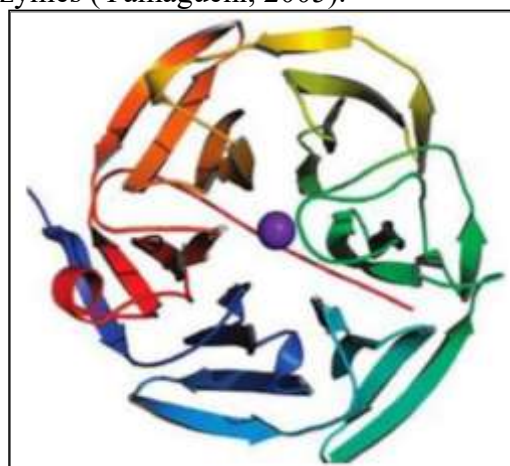


Figure (1): showing crystal structure of human Regucalcin (RGN) with calcium (Ca^{2+}) bound, (Adapted by Chakraborti and Bahnson, 2010).

Previous studies, with rat and mouse RGN, have reported that Zn^{2+} is the metal of choice in gluconolactonase activity and Ca^{2+} -in cell regulation and homeostasis (Chakraborti and Bahnson., 2010). In rat tissues, Northern blot and immunohistochemical analyses showed

that RGN was specifically expressed in the liver and kidney, where its immunoreactivity was localised in the hepatocytes (in the nuclei and cytoplasm) and the renal tubular epithelia (Ishikawa et al., 2012). It is expressed in other rat tissues, namely, brain, lung, epidermis, stomach, adrenal gland, ovary, testis, mammary gland and prostate (Maia et al., 2008)

In human tissues, RGN was moderately expressed in the pancreas and heart, in addition to the expression in the liver and kidney (Kondo et al., 2008). RT-PCR and immunohistochemical analyses showed that RGN mRNA and protein were expressed in the cytosol and nuclei of breast and prostate epithelial cells (Maia et al., 2009).

In livers of rats, RGN decreases androgen-independently with ageing. Northern blot analysis showed a marked increase of RGN mRNA in livers of neonatal and young rats. The substantial amounts of protein and transcript were maintained in adults up to 3-6.5 months of age (Son et al., 2008). In the kidney, RGN mRNA and protein started to increase at day 21 and reached near-maximal levels at day 35. The levels of transcript and protein remained high in adults up to 3M of age (Yumura et al., 2006). As the ageing process progressed to senescent stages, the levels of transcript and protein decreased significantly in the liver and kidney of aged rats. The age-associated decrease of RGN in the liver and kidney may be in a large part controlled at transcriptional levels (Handa et al., 2009). The high expression of RGN in the tissue-maturing process and adult suggests that RGN may be required for the maintenance of highly differentiated hepatic and renal functions (Hasegawa et al., 2010).

FUNCTION OF REGUCALCIN: ROLES OF RGUCALCIN IN CALCIUM MAINTENANCE:

Regucalcin was initially discovered in 1978 as a regulatory protein in calcium signalling. That has been shown to play a pivotal role in cell regulation: maintaining of intracellular calcium homeostasis, suppressions of signal transduction, inhibition of translational protein synthesis, nuclear deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis,

regulation of gene expression, and anti-effects on proliferation and apoptosis in many cell types (Yamaguchi., 2013c).

Regucalcin is translocated from the cytoplasm to nucleus of cells, and also it binds to the mitochondria and microsomes. Regucalcin has been shown to suppress Ca²⁺ signalling, which is involved in various Ca²⁺ calmodulin-dependent and independent protein kinases and protein phosphatases. Moreover, regucalcin plays a pivotal role in cell regulation: maintaining of intracellular Ca²⁺ homeostasis (Marques et al., 2014a).

REGUCALCIN ANTI-APOPTOTIC EFFECT:

Regucalcin also has a suppressive effect on various signaling pathways from the cytoplasm to nucleus in proliferating cells and regulates nuclear function in including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis and the overexpression of endogenous regucalcin was found to suppress apoptosis in modeled rat hepatoma cells and normal rat kidney proximal epithelial cells induced by various signaling factors (Harliansyah, 2016). Suppressive effect of regucalcin on apoptosis is related to inhibition of nuclear Ca²⁺ activated DNA fragmentation, Ca²⁺ calmodulin-dependent nitric oxide synthase, caspase-3, Bax, cytochrome C, protein tyrosine kinase, protein tyrosine phosphatase in the cytoplasm and nucleus. Moreover, regucalcin stimulates Bcl-2 mRNA expression and depresses enhancement of caspase-3, Apaf-1 and Akt-1 mRNAs expression (Goldar et al., 2015).

A high concentration of NO, which is produced by inducible NO synthase, has been shown to suppress cell proliferation and induce cell apoptosis (Monier and Suzanne, 2015). It has been reported that a low concentration of NO, which is produced by endothelial NO synthase, protects against cytotoxic effects of reactive oxygen species in cells (Tower, 2015). Whether endogenous regucalcin suppresses NO production in H4-II-E cells is unknown at present, although it inhibits NO synthase activity (Shalini et al., 2015). It is speculated, however, that regucalcin depresses NO production in H4-II-E cells, endogenous regucalcin may have the anti-apoptotic effect

due to suppressing NO production in hepatoma cells (Correia et al., 2016).

Moreover, overexpression of regucalcin has been shown to have a suppressive effect on TNF- α -induced apoptosis in human hepatoma HepG2 cells (Rahbar et al., 2015). Akt, which is a survival factor in cells, has been shown to activate in transfectants (Nakagawa and Yamaguchi, 2008).

Regucalcin suppresses lipopolysaccharide (LPS)-induced apoptosis. LPS induces cell apoptosis (Chakraborti and Bahnsen, 2010). LPS causes a decrease in the number of H4-II-E cells (wild-type), inducing cell death and apoptosis (Nikapitiya et al., 2008). This decrease was completely protected by overexpressing of endogenous regucalcin with culture for 12–48 h (Arun et al., 2011). Thus, overexpression of endogenous regucalcin has suppressive effects on LPS-stimulated cell death and apoptosis. Regucalcin suppresses on calcium signaling-induced apoptosis, Calcium channel blockers, the endoplasmic reticulum Ca²⁺ ATPase inhibitor thapsigargin and calcium ionophores are potent to lead several cell types to apoptosis (Justet et al., 2016). Thapsigargin is an inhibitor of Ca²⁺ ATPase in the endoplasmic reticulum (Ca²⁺ store) in cells, and treatment with thapsigargin causes an elevation of sustained Ca²⁺ concentration in cells and induces apoptosis in hepatoma cells (Orrenius et al., 2015; Vaz et al., 2016).

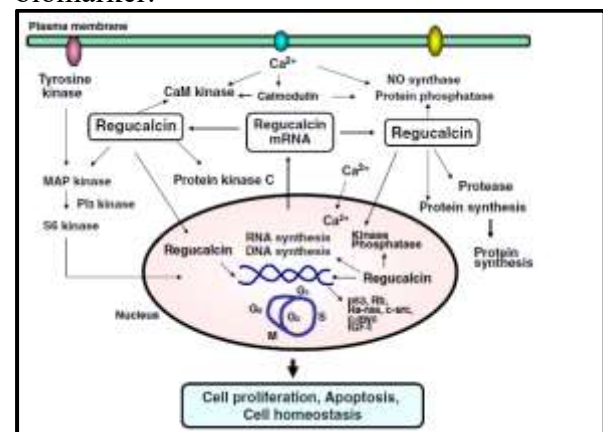
Regucalcin as Biomarker in Various Disease:

The expression of the regucalcin gene and its protein has been shown to alter with various metabolic diseases, and regucalcin plays an important role in the development of many pathophysiologic states. Serum regucalcin has been found to increase with liver injury, and also urinary regucalcin is elevated with kidney damage, suggesting a useful tool as a biomarker for diagnosis (Yamaguchi, 2010). Moreover, regucalcin has been shown to be a good tool in early diagnosis of Alzheimer's disease and other brain diseases (Kim et al., 2010b).

Regucalcin has been demonstrated to play a pivotal role in cell regulation in various tissues and cell types (Elchuri et al., 2007). Regucalcin is translocated from the cytoplasm to nucleus of cells with a mechanism of

calcium signalling system, and the cytoplasmic regucalcin can bind to the mitochondria and microsomes (Park et al., 2010a). Regucalcin has been shown to suppress the activity of various protein kinases and protein phosphatases due to binding to their proteins and calmodulin (Lv et al., 2008). Moreover, regucalcin plays a pivotal role in cell regulation: maintaining of intracellular Ca²⁺ homoeostasis. Suppression of protein synthesis at the translational process (Yamaguchi et al., 2008).

There is growing evidence that regucalcin may be a key molecule which is related to the development of various diseases, regucalcin has been shown to play a pathophysiologic role in the disorders of liver and kidney, in which the protein is greatly expressed as figure (2) (Nakagawa and Yamaguchi, 2005). Furthermore, regucalcin has been found to involve in lipid metabolism, diabetes, and osteoporosis (Arbillaga et al., 2008). Thus, it has been suggested that regucalcin is involved in disease. Estimation of regucalcin in disease condition may be a diagnostic significance as a biomarker.



Fig(2): showing regucalcin has been demonstrated to play a pivotal role in cell regulation in various tissues and cell types (Klawitter et al., 2010).

REGUCALCIN IN LIVER DISEASE:

Regucalcin is remarkably expressed in the liver among various tissues. The concentration of regucalcin was estimated to be 80 and 52pg in the livers of male and female rats, respectively. Regucalcin over 2 % of total proteins is expressed in liver cells (Vaz et al., 2016).

Regucalcin plays a multifunctional role as a suppressor protein in signal transduction in cell regulation (Marques et al., 2014b). Hepatic regucalcin mRNA and its protein expressions have been shown to suppress with liver damage and disorder, suggesting an involvement of regucalcin in the development of liver disease (Yamaguchi, 2015). Regucalcin has been found to be connected with the development of hepatocellular carcinogenesis and considered as new markers for the liver preneoplastic foci in rats treated with diethylnitrosamine and 2-acetylaminofluorene after partial hepatectomy which induces an increase in proliferating cells. Suppression of regucalcin gene expression was seen from the early stage in the development of carcinogenesis and this suppression is suggested to take a part in the development of carcinogenesis in liver cells (Williams et al., 2016).

The suppression of regucalcin expression accelerates the enhancement of various signalling pathways in liver cells, it may generate a possible circumstance for the development of carcinogenesis. In this aspect, the suppression of the regucalcin gene in carcinogenesis with chemical feeding in vivo may play a pathophysiological role in the development of carcinogenesis (Williams., 2010). Moreover, regucalcin has been identified to be biomarkers associated with the development of hepatocellular carcinoma in superoxide dismutase-deficient mice in vivo (Harikrishna et al., 2016). Regucalcin showed a divergent alteration in samples. Whereas elevated regucalcin levels were observed with no obvious neoplastic changes, a marked reduction in regucalcin is observed with fully developed hepatocellular carcinoma (Marques et al., 2014b).

The decrease in regucalcin, which plays a role as a suppressor in various signalling pathways in proliferating cells (Davis et al., 2015), may lead to development in carcinogenesis. Overexpression and deficiency of endogenous regucalcin in the liver have been found to induce a disorder of lipid metabolism in the liver (Pardo and Stuhmer, 2014). Overexpression of regucalcin stimulated glucose utilisation and lipid

production in liver cells, inducing hyperlipidemia (Okada et al., 2015).

Interestingly, involvement of regucalcin in insulin resistance has been shown in modelled rat hepatoma cells in a culture system with the use of the cytokine tumour necrosis factor-alpha (TNF-a) and insulin (Lai, 2010), which mimics insulin resistance in human type-2 diabetic Mellitus. Regucalcin has been reported to be implicated in the pathogenesis of the nonalcoholic fatty liver disease, therefore hepatic regucalcin was decreased with a fibrosis and an increase in the serum low-density lipoprotein (Laakso and Kuusisto, 2014). The level of regucalcin dependent on the stage of disease was changed. There may be a correlation between regucalcin levels and disease prognosis. However, it is not known whether decreased hepatic regucalcin is a result or a cause of cirrhosis (Arbillaga et al., 2008).

The function of regucalcin in the liver fibrosis has been explored in regucalcin knockout mice and this In vivo effect was confirmed using isolated hepatic stellate cell (HSC). In carbon tetrachloride (CCL4)-induced liver fibrosis, the nuclear translocation, which is downstream in signalling transduction of transforming growth factor beta (TGF-b), was found to be significantly inhibited in the liver of regucalcin knockout mice (Zhang et al., 2014). Suppression of hepatic regucalcin may partly lead to the liver fibrosis through enhancement of PPAR-gamma expression (Qu et al., 2014).

It has been examined whether serum regucalcin has a potential sensitivity as a specific biochemical marker of chronic liver injury in rats orally administered with a comparatively lower dose of CCL4, among various serum biochemical markers for hepatitis, the change in serum regucalcin was a potential for chronic liver damage in rats (Kim et al., 2015). Moreover, regucalcin is found to release in the serum of human subjects with hepatitis, and regucalcin was found in the serum of patients with chronic liver injury, although the protein was not detected in the serum of normal subjects (Kadhim, M. M. Aldujaili, A. N. and Homady, M. H., 2017).

Regucalcin is stable for temperature and is not decomposed with the freeze for longer

periods, the determination of serum regucalcin was valuable as a diagnostic tool for chronic liver injury with a comparatively lower level of serum GOT and GPT activities, Thus, serum regucalcin may be a potential sensitivity as a biochemical marker for hepatitis (Callegari et al., 2013).

Finding, that regucalcin is important as a potential biomarker for the diagnosis of liver failure, has also been supported by studies in the plasma and liver tissues of mice and humans with acute liver failure using a two-dimensional electrophoresis (2-DE), mass spectrometry proteomics assay, Western blot, and real time-PCR analysis, and these data validated that regucalcin protein levels were

only elevated in the plasma of mice with acute liver failure (Purohit et al., 2013). Further analysis revealed that D-galactosamine/lipopolysaccharide (GalN/LPS) induced the downregulation of regucalcin protein levels in the liver tissues 25 and 16% in the GalN/LPS-treated mice and in the treated mice that survived, respectively (Amano et al., 2014).

The study of (Yamaguchi, 2014a) supports the view that regucalcin is a potential biomarker for the diagnosis and prognosis of acute liver failure. Regucalcin may play an important role in a self-protective mechanism in survival, and participates in the pathophysiological processes of acute liver failure Table (1).

Table (1): showing regucalcin as an indicator of several cases diseases.

| Disease | Tissue or sample | Regucalcin change | Refs. |
|----------------------------------|------------------|-------------------|---------------------------------|
| Liver injury | Liver | Decrease | (Lv et al., 2008) |
| Carcinogenesis | Liver | Decrease | (Elchuri et al.,2007) |
| Insulin resistance | Liver | Decrease | (Nakashima and Yamaguchi, 2007) |
| Nonalcoholic fatty liver disease | Liver | Decrease | (Park et al., 2010) |
| Kidney damage | Kidney | Decrease | (Klawitter et al., 2010) |
| | Urine | Increase | |
| Hepatitis | Serum | Increase | (Lv et al., 2008) |
| Hypertension | Kidney | Decrease | (Nakashima and Yamaguchi, 2007) |
| Alzheimer's Disease | Serum | Increase | (Yamaguchi, 2014b) |
| Parkinson's disease | Brain | Increase | (Van et al., 2012) |

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