

Effects of Plaferon LB (PLB) on concanavalin A-induced hepatitis in mice

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Abstract

PLB is a mixture of human placental proteins possessing immunomodulatory, antihypoxic and other possibilities. The present study evaluated the effects of PLB on ConA induced hepatitis in mice. Dexamethasone as a control drug was used. After 4 h from ConA injection drugs were administered intramuscularly. PLB as well as dexamethasone significantly decreased the activity of serum ALT activity after 8, 24 and 48 h from ConA administration. In histological investigation, at the time point of 48 h from ConA administration in mice treated with PLB spotty necrosis accompanying inflammatory cell infiltration was not observed. ESR signals of MetHb, xanthine oxidase (XO) and other indicators of the generation of free radicals in the liver tissue were significantly lower in mice treated with PLB compared with controls, while nitric oxide level was kept in high index. The latter protects hepatocytes from apoptotic death. These results suggest that ConA induced hepatitis was ameliorated by treatment with PLB. With regard to the effects of PLB, we speculate that mechanism of PLB influence on immune-mediated tissue injury depends on the inhibition of the inflammatory cytokines production as well as the generation of free radicals.

Keywords: *ConA induced hepatitis, oxidative stress, Plaferon LB*

Introductions

Although the mechanism underlying the onset of liver injury such as viral hepatitis or autoimmune hepatitis is unclear, mainly T-cell immunity is considered to intervene in the mechanism, which is thought to be regulated by various cytokines (8). As detailed clinical studies are difficult, diverse models of experimental liver injury have been advocated. In this models, drugs with hepatotoxicity or endotoxin were mainly used.

ConA is a lectin which stimulates lymphocytes or monocytes to secrete various cytokines in vitro, and it's known to induce liver injury mediated by cellular

immunity injected into mice (8). The ConA induced liver injury model and clinical liver injury seem similar (8).

In case of ConA-induced experimental liver injury in mice, the so-called inflammatory cytokines such as TNF-alpha and IFN-gamma were also reported to be responsible for the onset (13). As it's already known IFN-gamma promotes the expression of iNOS (16).

The disbalance of oxidative-antioxidative system plays also a main role in developing of pathogenesis of ConA induced hepatitis (12).

The search of protective substances against immune-mediated liver injury is an actual problem of

investigation. It is shown that aminoguanidine, soyasapogenol A, glycyrrhizin, dexamethasone, cyclosporine A etc. prevent liver injury by different mechanisms (9, 10, 15). But today it is not known effective remedy for treatment of immune-mediated liver injury in clinic.

Due to pathogenesis of the disease, some authors recommend to include the immunotropic drug into the scheme of therapy (3, 6, 14).

It's known that PLB expresses immunomodulatory and antiinflammatory effects: it inhibits mitogen induced proliferative activity of peripheral blood lymphocytes and IFN-gamma production by antigen-stimulated cells (4). PLB reduces NO production by LPS-stimulated macrophage/monocyte cells (1) and also protects the metabolic and morphologic changes in the liver tissue in cases of various pathologies (2). That's why we decided to investigate PLB influence on liver in case of Con A dependent hepatitis. In the previous study dexamethasone was used as a control drug.

Material and methods

Experiment was conducted on 27 white mice weighing 20-25g.

Preparation of experimental model

ConA, purchased from Serva, USA (2mg/kg) was dissolved in saline and injected intravenously in a volume of 10ml/kg. Mice were divided into three groups. After 4 hours from ConA administration the animals were injected by 0,2 ml saline (first group), 0,25 mg/kg PLB (second group) or 5mg/kg dexamethasone (third

group). The latter served as a control drug.

8, 24, 48 h later from ConA injections the anesthetized animals were sacrificed by cervical dislocation. Blood was sampled and at each time point, the serum ALT level was measured by spectrophotometry" Aminotransferase ALT-AST (AST-ALT-180)".

For morphological study the liver tissue was fixed in 12% formalin and embedded in paraffin. Subsequent hematoxylin and eosin (H&E) staining of the preparation was followed.

For determining the oxidative processes in the liver tissue mitochondrial respiratory chain condition, activity of XO, content of MetHb and NO were measured. Tissue was placed in polyethylen tubes and kept in liquid nitrogen. Masses of specimen were 200-300 mg. The oxidative processes in the liver were studied by ESR spectroscopy method on a Radiospectrometer RE-1307 (Russia). For fixation of unstable molecule of nitric oxide NO-trap (Na-diethyldithiocarbonate, Pharmachim, Moscow) was used.

Results

Biochemical measurement

Biochemical measurements were performed using sera obtained from mice 8, 24 and 48 hours after ConA administration. As shown in Fig.1 ALT levels in mice from II and III groups were significantly lower than those in mice from group I.

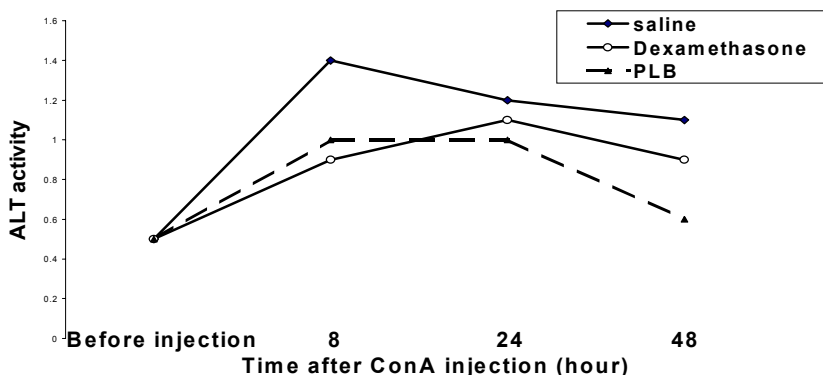


Fig.1 Serial changes in serum ALT activity after the ConA injection..

Histological Examination

After 8 h from ConA administration in liver tissue of all mice the hepatocytes undergo hydrophic degeneration, necrosis of hepatocytes is also characteristic.

At the time point of 24 h of investigation the significant morphological differences in the liver tissue of I, II and III group animals is not observed; degenerative changes become more severe, most of hepatocytes undergo vacuolar dystrophy.

At the same date of 48 h from ConA injection, in the liver tissue of the first group mice still most of hepatocytes undergo dystrophic and degenerative changes. Spotty necrosis still exists. In some areas the normal trabecular structure of liver is destroyed (Fig.2).

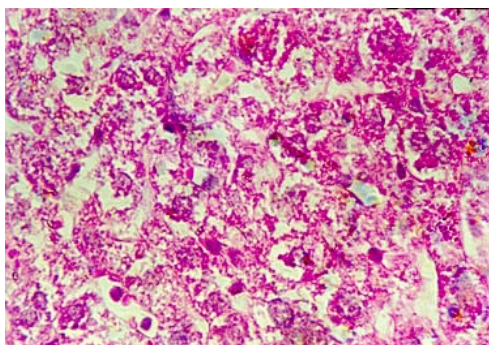


Fig.2 Liver after 48 hours from the ConA injection (saline).

At the same time of investigation under the influence of a control drug, dexamethasone, the normal structure of liver, in most regions is restored, although still exists regions where hepatocytes undergo hydrophic and necrotic changes (Fig.3).

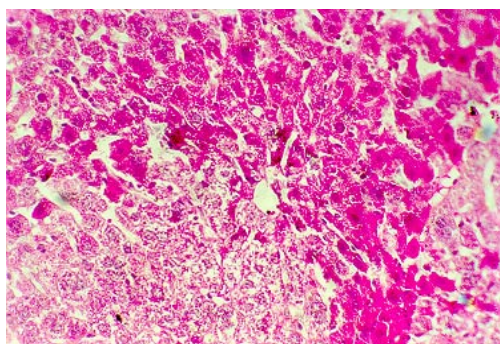


Fig.3 Liver after 48 hours from the ConA injection (Dexamethasone)

At the same date (48h), under the influence of PLB the morphology of liver tissue is significantly improved in comparison with the liver histology of the second group mice; generally the normal structure of the liver tissue is restored, the areas of destruction and necrosis are not characteristic (Fig.4).

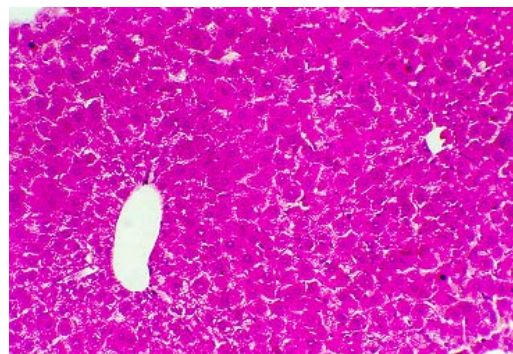


Fig.4 Liver after 48 hours from the ConA injection (PLB).

ESR-measurement

Tab.1 illustrates ESR changes in the liver tissue in case of Con A-induced hepatitis. The condition of mitochondrial respiration chain was evaluated by I ($g=2,0$) ESR signal and its DH index. Significant reduction of I ESR signal and its DH index in control group show the disturbance of mitochondrial respiratory chain due to the electron transport failure on the NAD.H-oxidoreductase region. This indicates the presence of plenty amount of oxygen free radicals generator - semiquinones.

PLB as well as dexamethasone keeps DH index in its normal level at all time points of investigation.

For determining the activity of another free radical generator - XO the ESR signals of Mo5+ was studied. Appearance of this signal in case of ConA induced hepatitis is a marker of XO activation because containing of Mo5+ in its active part. This index was significantly decreased under the influence of PLB and dexamethasone at the 8 and 24 h, and absolutely disappeared at the end of the experiment in mice treated with PLB.

Detectable ESR signal of MetHb in liver tissue in case of immune-mediated liver injury also indicates oxidative stress. MetHb keeps its high level in the control mice up to the end of the experiment. Under the influence of dexamethasone and PLB the same index is significantly decreased. The latter was more expressed in case of PLB.

| Group | Hour | Mitochondrial respiratory chain | | XO | MethHb | NO |
|------------------------|------|---------------------------------|----------|----------|----------|----------|
| | | I | ΔH | | | |
| Norm | | 36 | 11 | - | - | 8,6 |
| First (saline) | 8 | 35 | 7,6±0,5 | 12.3±2,5 | 21±6,5 | 20 |
| | 24 | 34±0,5 | 8±0,2 | 15±0,2 | 19±2 | 40±0,2 |
| | 48 | 26,6±2,6 | 6,3±0,5 | 8±1,5 | 20,6±0,9 | 51±1,5 |
| Second (Dexamethazone) | 8 | 68±0,2 | 11,6±0,5 | 8±1 | 12,3±2,5 | 36,6±4,5 |
| | 24 | 50±0,2 | 10,9±0,9 | 7±0,2 | 15±0,2 | 50±0,2 |
| | 48 | 45±5 | 12±0,2 | 11±1 | 18,3±1,5 | 36,6±4,5 |
| Third (PLB) | 8 | 30±0,2 | 13±0,2 | 8.,3±1,5 | 10±0,2 | 35,6±5,4 |
| | 24 | 35±5 | 12±0,2 | 7,6±0,5 | 8,6±0,8 | 30±0,2 |
| | 48 | 30±0,2 | 12±0,2 | 7,0 | 6±1 | 42,5±2,5 |

Tab.1 Serial changes of various indices in liver tissue after the ConA injection..

By using NO-trap NO ESR signal in the liver tissue was measured. At the 8 and 24 h NO index in the control mice was lower in comparison with the II and III group animals. Under the influence of PLB and dexamethasone the synthesis of nitric oxide was significantly intensified 48 h later from Con A injection.

Discussion of Results

Con A, a kind of lectin, binds sugar residues on the surface of a wide variety of different cell types and stimulates T lymphocytes and macrophages. ConA facilitates cellular immunity in liver tissue, thereby inducing liver injury (8).

Mice with liver injury induced by ConA are, therefore used as experimental models mediated by cellular immunity. Interestingly, the activated lymphocytes start to infiltrate the liver tissue 8 h after ConA injection, after liver damage has already begun, the maximal levels of the most cytokines were reached before infiltration of lymphocytes occurred. TNF-alpha (13) and IFN-gamma (16) have been shown directly contribute to liver cell damage; IFN-gamma promotes the expression of iNOS and TNF-alpha induces apoptosis (16).

The development of ConA induced hepatitis and influence of PLB was estimated by detecting dynamics of serum AlAT activity and histological changes in the liver tissue.

Treatment by PLB as well as by control drug-dexamethasone result in significant decrease of serum AlAT level and improved histological appearance of the liver.

These results suggest that PLB possesses treatable effect in case of liver injury.

With regard to the etiology of ConA induced hepatitis various studies have been performed focusing on the analysis of cytokine production. TNF-alpha and IFN-gamma are the most important agents - inflammation inducers and IL-10 is also an important factor that controls inflammatory processes (7). As the previous studies revealed the inhibitory effect of PLB on IFN-gamma production by mitogen stimulated lymphocytes (4), we suggest that one of the protective mechanisms of PLB (in present study) can be the inhibition of IFN-gamma production after ConA injection.

IFN-gamma is an inducer of iNOS expression (16), which contributes the plenty amount of nitric oxide.

Under the influence of PLB and dexamethasone the positive correlation between dynamics of serum AlAT and restoration of the liver structure was observed in case of ConA induced hepatitis, but at the same time NO was kept in its high level. Its content in the liver tissue of mice treated with PLB is significantly higher at 48 h of investigation in comparison with the same index of the first group animals. As one of the free radicals, the damage effects of NO is well known, despite this nowadays it's already revealed that NO protects hepatocytes from TNF-alpha induced caspase 3 and caspase 8 and accordingly from the apoptotic death (5, 11).

Thus, elevation of NO under the influence of PLB and dexamethasone probably is a result of the compensative changes in ConA induced hepatitis. Due to the data of inhibition of iNOS by PLB and

dexamethasone (1), we speculate that elevation of NO under the influence of these drugs in the present study is a result of intensification of constitutive NOS.

According to our results, PLB decreases the ESR signals of XO, which catalyzes the removal of hydrogen from substrate using oxygen as a hydrogen peroxide as a result of reaction.

We revealed that activity of XO and total amount of free radicals are subsequently decreased in mice treated with PLB, accordingly the low intensity of ESR signals of MetHb was proved by our experiment.

Thus, the protective effect of PLB on metabolic changes in liver tissue is a result of inhibition of XO and restoration of electron transport in mitochondrial respiratory chain.

References

1. Gongadze M.T., Chikovani T.I., Saniidze T.V., Bakhutashvili V.I. The influence of Plaferon LB on the LPS-induced synthesis of NO. // *Int. J. Immunorehab.* V.3 N3 2001. P 158-160.
2. Chikovani T., Rukhadze R., Pantsulaia I., Sanikidze T., Bakhutashvili V. (1999). // *Intern J Immunorehabilitation* 12S, 14-19
3. Im EH., Lee BS., Sung JK., Lee SO., Lee KT., Lee SM., Kim SH., Seo KS., Kim JH., Kim SG., Kim NJ., Lee HY // *Korean J Intern Med* 1999 14(1):1-8
4. Khetsuriani N., Chikovani T, Alies Snieders // Institute of medical Biotechnoogy, Tbilisi, Georgia.Laboratory of cell biology,University of Amsterdam, The Netherlands (unpublished data)
5. Li J., Bombeck C.A., Yang S., Kim Y.-M., Billiar T.R. *J. Biol Chem.*274:17325-17333, 1999
6. Liaw Y., Tsai S., Chein R., Yeh C., Chu C. // *Hepatology*, Vol.32, No.3, 2000, p.604-609
7. Luis H, Le Moine O, Peux Mo, Quertinont E.*hepatology* 1997 Jun;25(6)
8. Mitsuro Kato, naoki Ikeda, Eiki matsushita, Shuichi Kaneko, Kenichi Kobayashi // *hepatology research* 20 (2001) 232-243
9. Okamoto T, Masuda Y, Kawasaki T, Shinohara M, Matsuzaki K. Aminoguanidin prevents concanavalin A - induced hepatitis in mice.// *Eur J Pharmacol* 2000 May 19;396(2-3):125-130.
10. Okamoto T, Yamamura K, Hino O. Expression of the inducible form of the nitric oxide synthase gene in the livers of mice with chronic hepatitis.//*Int J. Mol Med* 2000 Sep; 6 (3):315- 317.
11. Rai M., Lee F. Y. J., Rosen A., Yang S. Q., Lin H.Z., Koteish A., Liew F.Y., Zaragoza C., Lowenstein C., Diehl A.M. *Proc. Natl.Acad.Sci.USA* 95:13829-13834,1998.
12. Satoh M, Kobayashi K, Ishii M, Igarashi T, Toyata T. Midsonal necrosis of the liver afer concanavalin A-injection. Third Department of Internal Medicine, Tohoku University School of Medicine, Sendai. 83 // *J. Exp. Med.* 1996. N180(2). P. 139-52.
13. Tagawa Y, Sekikawa K, Iwakura Y. Suppression of con A-duced hepatitis in INF-gamma (-/-) mice, but not in TNF alfa (-/-) mice: role for INF-gamma in activating apoptosis of hepatocytes. // *J. Immunol.* 1997. 1. 159(3). P. 1418-28.
14. Tam RC., Pai B., Bard J., Lim C., Averett DR., Phan UT., Mulovanovic T. // *J Hepatol.* 1999, 30 (3):376-82
15. Tiegs G, Hentschel J, Wendel A. T-cell dependent experimental liver injury in mice indusible by concanavalin A. // *J. Clin. Invest.* 1992. N90(1). P. 196-203.
16. Trautwein et al.// *J Clin. Inves.* Volume 101, No 9, May 1998

Влияние плаферона ЛБ (PLB) на конканавалин А (Con A) индуцированный гепатит

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Р Е З Ю М Е

Нами исследованы сравнительные защитные возможности иммуностропных препаратов PLB и дексаметазона при Con A-индуцированном гепатите. Для воспроизведения экспериментальной модели гепатита 27 мышам внутривенно ввели Con A (SERVA, USA) в дозе 2 мг на кг веса. Через 4 часа после индуцирования гепатита, мышам, которые составляли контрольную группу, вводили физиологический раствор, второй группе - PLB в дозе 0.25 мг/кг., а третьей - дексаметазон в дозе 5 мг/кг. Инъекции повторялись через 24 часа. Спустя 8, 24 и 48 часов после начала эксперимента животные забивались методом цервикальной дислокации под эфирным наркозом. В крови активность аминотрансферазы определяли спектрофотометрическим методом. Для гистологического исследования парафиновые срезы ткани печени окрашивали гематоксилин-эозином. С целью определения окислительных процессов в печени спектры ЭПР образцов печени регистрировали на радиоспектрометре РЭ-1307. Было выявлено, что при Con A-индуцированном гепатите PLB и дексаметазон уменьшают активность аминотрансферазы, способствуют восстановлению нормальной структуры печени и уменьшают активность окислительных процессов в печени. Защитный эффект был более полноценным при использовании PLB.

Ключевые слова: ConA индуцированный гепатит, окись азота, плаферон ЛБ, окислительный стресс