

Effect of Plaferon LB in Ischemia/Reperfusion of the Pancreas

*Rusudan Rukhadze, Tamar Sanikidze, Malkhaz Mirtskhulava,
Manana Papava*

TSMU Scientific Research Institute of Experimental and Clinical Medicine, Georgia

Abstract

The aim of the present study was to find out whether plaferon LB, a well-known antioxidant and membrane stabilizer could be applied in pancreas preservation for protection pancreatic tissue from cell damage, which occurs during ischemia/reperfusion. To evaluate the protective effect of plaferon LB part of animals received widely used protectors a tocopherol, unithiol and vitamin C. According to received data plaferon LB appears to protect the pancreas from ischemia reperfusion injury by reducing the peroxidation of the pospholipids and maintaining the activity of SOD more effectively, than a tocopherol, unithiol and vitamin C together.

Keywords: *pancreas, ischemia reperfusion, plaferon LB*

Introduction

Tissue damage as a consequent of ischemia is a major medical problem in an industrialized society [1]. The pancreas is an organ highly susceptible to ischemic damage. Post-ischemic injury is one of the most important problems affecting successful organ procurement and transplantation. Ischemia as a causative factor for acute pancreatitis has been discussed for decades, but has only recently gained wider acceptance. Whereas the conventional view has attributed the injury process to ischemia itself, recent studies have found that a variable, but often substantial proportion of the injury is caused by toxic oxygen metabolites. Oxygen free radicals, activation of polymorphonuclear leukocytes, failure of microvascular perfusion, cellular acidosis and disturbance of intracellular homeostasis appear to be important factors/mechanisms in the pathogenesis of ischemia/reperfusion induced acute pancreatitis [2].

Temporary pancreatic ischemia, with following reperfusion and oxygen-derived free radicals appear to play an important role in the development of graft pancreatitis and thrombosis after pancreas transplantation. Up to now there are few therapeutic

options for this severe complication, because very little is known about pancreatic ischemia/reperfusion injury [3]

Several antioxidants, free radical scavengers and protease inhibitors (such are SOD, thromboxane, a-tocopherol, lidocaine, unithiol, etc.) have been used as possible protective agents against ischemia/reperfusion injury. [4,5,6]

The purpose of the present study was to find out whether plaferon LB, a well-known antioxidant and membrane stabilizer [7] could be applied in pancreas preservation for protection pancreatic tissue from cell damage, which occurs during ischemia/reperfusion.

Materials and Methods

Experiments were performed on 62 adult (200-250g body weight) rats. The cold ischemia of the pancreas using chlorethyl was induced by S.A.Shalimov method (1989). Ischemia of 20 min duration was followed by reperfusion. Animals were divided into three experimental groups. 20 animals form the control group. Rats of the second group (16 animals) every day during three days and additionally 3 hours before start of cold

ischemia were injected by plaferon LB (0,35 mg per kg body weight). To evaluate the protective effect of plaferon LB animals of the group III (16 animals) received the injection of a tocopherol, unithiol and vitamin C. The animals of the control (I) group and intact rats (10 animals) at the same steps of experiment received 0,2 ml of saline.

The effect of ischemia/reperfusion on the pancreas and the possible protective action of plaferon LB were assessed by light microscope and ESR study.

Rats were sacrificed under the ether narcosis and tissue samples were taken 20 minutes after start of cold ischemia and 40 min after reperfusion.

Tissue samples for light microscopy were fixed in the 10% solution of phormaldehyde, with following dehydration and embedding in the paraffin. Tissue sections were stained by hematoxilin and eosin.

For ESR study tissue samples of pancreas were placed in polyethylene tubes and kept in liquid nitrogen. The masses of specimen were 200-300 mg. ESR measurements were performed on a radiospectrometer RE-1307 (Russia). Samples were placed into a standard quartz flat cell. ESR specter of free radicals and their semiwidth, iron-sulphurous complexes (FeS) ($g=2,03$), Fe^{2+} , nitric oxide (NO) and Mn^{2+} containing complexes ($g=2,15$), were determined.

Results and Discussion

The present findings indicate that transient pancreatic ischemia leads to the development of acute necro-haemorrhagic pancreatitis. The Hemorrhages and intravasal coagulations are typical findings. The overall histological tissue injury was most severe in the control group. Postischemic reperfusion was associated with further increase of microcirculatory damage and large hemorrhagic lesion formation. In ischemia/reperfusion of the pancreas impairment of microcirculation after reperfusion is believed to be of critical importance. The 'no-reflow' phenomenon is thought to cause persisting tissue ischemia while the 'reflow-paradox' is defined as secondary impairment of nutritive perfusion. The data show a relevant impairment of tissue oxygenation after reperfusion.

Ischemia/reperfusion injury is characterized by a disturbance of the pancreatic microcirculation exhibiting a correlation with the duration of cold ischemia [8]. Although we haven't studied the results of cold ischemia of different duration, but our data indicate that even the short termed cold ischemia with following reperfusion cause the serious damage of the organ.

Hemorrhagic lesions correlate with well-demarcated patchy lesions composed of degenerating acinar cells

showing deeply eosinophilic cytoplasm and piknotic nuclei, indicating fresh ischemia. In the marginal zone of the larger lesions and in small lesions the intralobular ductules have avoided the ischemic changes. The acinar necrosis was most obvious at the periphery of the lobules.

Under the action of a tocopherol, unithiol and vitamin C the hemorrhagic lesions were less prominent. the lobular structure of the pancreas is retained, but in the middle of most of lobules the degenerating acinar cells, with deeply eosinophilic cytoplasm and picnotic nuclei were observed. Despite the protective effect of the therapeutic agents, reperfusion augmented significantly the severity of findings.

The rate of morphologic changes was minimal in both ischemia/reperfusion in the group of animals, who received the injection of Plaferon LB.

To understand the molecular basis of histological lesions the ESR study of pancreas was performed. The data obtained by ESR study are summarized in *Tab. 1*.

As it is seen from the table during 20 min cold ischemia of the pancreas the intensity of free radicals ESR signal is significantly decreased and continues to decrease after reperfusion. The intensity of iron-sulphurous proteins' (FeS) ESR signal never changes during ischemia and increases significantly after reperfusion. The observed changes indicate the decreased restoration rate in mitochondrial electron system and the subsequent formation of large amount of byproduct ubisemichinone - a powerful source of free radicals. An alteration of the pancreatic energy metabolism may be an important pathogenic factor in ischemia/reperfusion injury of the pancreas. On the other hand, as was mentioned above, free radicals are supposed to be instrumental in the development of ischemic cell injury.

Under the action of plaferon LB, as well as under the influence of a tocopherol, unithiol and vitamin C, the intensity of free radicals ESR signal decreases, but is significantly higher, than the analogous parameter of the control group. In both cases (in the II and III groups) reperfusion isn't characterized by further decrease of the parameter.

The intensity of FeS ESR signal in both (II and III) groups was retained under the normal range, but this parameter increases after reperfusion in the group of animals, receiving a tocopherol, unithiol and vitamin C. Only plaferon LB retains the normal level of this parameter after reperfusion.

In all experimental groups the ESR signal of Fe^{2+} was detected. The ESR signal of this parameter is almost not characterized for the ESR specter of the pancreas of intact rats. Detection of this signal in the ESR specter

indicates the activation of oxidative processes and increased formation of free radicals.

The highest ESR signal of this parameter during ischemia/reperfusion was observed in the control group. Under the influence of plaferon LB and a tocopherol, unithiol and vitamin C intensity of Fe^{2+} ESR signal is approximately 1,5 times lower than in the control group.

So, plaferon LB, as well as a tocopherol, unithiol and vitamin C might be able to decrease the rate of oxidative stress and formation of free radicals. Our data indicate, that plaferon LB more effectively preserves the pancreatic energy metabolism and oxidative status of the cells.

The ischemia/reperfusion was characterized by increase in intensity of Mn^{2+} containing complexes ESR signal, that indicates the decreased activity of SOD - a key antioxidant enzyme.

MnSOD expression might reflect a defensive mechanism of accinar cells against oxidative stress. The measurement of MnSOD may prove useful as a marker of the effectiveness of treatment designed to limit ischemia/reperfusion injury [9].

a Tocopherol, unithiol and vitamin C retain this parameter at the normal level only during ischemia, whereas after reperfusion ESR signal of this parameter increases, indicating decreased activity of SOD. Plaferon LB retains the intensity of Mn^{2+} containing

complexes at the normal level during both, ischemia and reperfusion. Thus, Plaferon LB not only decreases the rate of free radical' formation, but also retains the antioxidative activity of pancreas at the normal level.

During ischemia/reperfusion a high ESR signal of nitric oxide (NO) was detected. The role of NO in the ischemia/reperfusion injury is still unclear. Recent data [9,10,11] suggest, that formation of NO in ischemia reperfusion associated with pancreas transplantation could modulate the inflammatory response. NO is generated and may have a protective role in ischemia/reperfusion although some authors indicate, that during reperfusion NO modulates lipoperoxidation levels and the development of pancreatic injury only when the ischemic period is quite short [12]. The ESR signal of NO was high in II and III experimental groups, but in both cases this parameter was significantly lower when compared with analogous parameter of the control group. According to our data, plaferon LB appears to protect the pancreas from ischemia reperfusion injury by reducing the peroxidation of the phospholipids and maintaining the activity of SOD. Plaferon LB preserves pancreas from injury not only during ischemia, but after reperfusion also and thus is more effective protector than widely used a tocopherol, unithiol and vitamin C together. The protective effect of later was apparent during ischemia, but was only slightly expressed after reperfusion. The improvement of molecular changes by plaferon LB was proved by histologic findings. The pancreatic tissue injury and hemorrhagic lesions were minimal during ischemia and don't impair greatly after reperfusion.

<i>Pancreas</i>	<i>n</i>	<i>Free Radicals</i>		<i>FeS</i>	<i>Fe²⁺</i>	<i>NO</i>	<i>Mn²⁺</i>
		<i>I</i>	ΔH				
Intact rats	10	25,45±0,7	13,6±0,3	8,39±0,09	—	11,9±0,2	5,35±0,2
Control group ischemia	10	18,35±1,62 *	11,22±0,6	6,04±0,07	51,37±1.15	35,34±0,65*	15,9±0,31*
Control group reperfusion	10	16,5±0,8 *	10,94±0,56	13±0,64	50,0±0,71	28,17±0,91*	17,16±1,9*
Plaferon LB + ischemia of pancreas	8	21,2±0,58*	13,3±0,29	7,1±0,95	31,13±0,71**	18,44±0,34*	8,06±0,74
Plaferon LB + reperfusion of pancreas	8	20,38±*0,75	12,88±0,33	10,1±1,0	30.17±0,6**	18,48±0,35*	7,13±0,43
α tocopherol, unithiol and vitamin C + ischemia of pancreas	8	20,2±0,42*	11,05±0,38	6,9±0,8	35,58±0,39**	19,96±0,38*	8,35±0,54
α tocopherol, unithiol and vitamin C + reperfusion of pancreas	8	18,2±0,42*	10,96±0,16	12,1±0,4 *	30,5±0,76**	18,72±0, 42*	11,2±0,6*

Tab.1 *The changes of pancreas ESR parameters during cold ischemia/reperfusion and after influence of plaferon LB, a tocopherol, unithiol and vitamin C*

*p< 0,001 compared with intact animals; ** p< 0,01 compared with control group

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Влияние плаферона ЛБ на панкреас при холодовой ишемии-реперфузии

Рухадзе Русудан, Саникидзе Тамара, Мирицхулава Малхаз, Папава Манана

Научно-исследовательский институт педиатрии, Тбилиси, Грузия

Р Е З Ю М Е

Целью данного исследования являлось выявление защитного эффекта отечественного препарата плаферона ЛБ на течение экспериментального панкреатита. Эффективность действия плаферона ЛБ сравнивали с эффективностью набора антиоксидантных препаратов: α -токоферола, унитиола, и витамина С, которые широко используются с целью антиоксидантной защиты при ишемии-реперфузии. На основании полученных данных можно заключить, что плаферон ЛБ способствует снижению интенсивности окислительных процессов и восстановлению активности антиоксидантных ферментов в панкреоцитах: уменьшается степень повреждения панкреаса как при ишемии, так и после реперфузии. Защитное действие α -токоферола, унитиола, и витамина С после реперфузии выражено незначительно.

Ключевые слова: *панкреас, реперфузионная ишемия, плаферон ЛБ*