APPLICATION OF DIFFERENT REMEDIES IN THERAPY OF EXPERIMENTALLY INDUCED AND CLINICALLY MANIFESTED SEPSIS

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Sepsis, as a systematic inflammatory reaction of an organism, still presents a big problem in therapy. Reanimation and supporting therapy, depending on time when the disease was recognized and further ways of treating, may influence on reducing mortality rate which ranges from 30 to 90%.

The process of reducing harmful consequences of septic condition is very complex. Besides usual therapeutic measures, this includes application of antibiotics, antishock therapy, antiinflammatory drugs, antagonists of endomorph system and postglands, satisfactory results are also obtained by application of antiendotoxin antibodies, antimonocine, anticomplementary antibodies and antioxidant remedies.

Investigation of new drugs is based on the principle of modification and prevention of undesirable metabolic, i.e. biochemic
and receptor cell activities. Using of these drugs finds its place in the experimental studies with good results. Above mentioned new drugs find their place in clinical praxis. When used on time, they guaranty success in therapy.

Key words: sepsis, therapy

INTRODUCTION

Sepsis, defined as a systemic response on infection, is characterized by a series of pathophysiological and clinical changes that include also change of body temperature, tachycardia, tachypnoea, leucopenia and leukocytosis. Besides all the knowledge in molecular biology and immunology, numerous aspects of pathogen changes in molecular and cellular level during sepsis are still unexplained.

It has been noted that an organism is not only an observer of dysfunctions caused by infective agents, but actively participates in this process by synthesis of mediators which, in the cascade of events, may lead to disfunction of certain organs, multiple organ failure (MODS) and death of the organism (BONE et al. 1987). The investigations performed on animals confirm partial influence of these mechanisms that are reflected in prolonged activation of complements, macrophagas, granulocyt aggregation in microcirculation and tissue lipid peroxidation on different organs. Through infective agent, activated cells secrete potentially poisoning mediators as cytokinines (TNF - tumor necrosis factor, IL-1 and IL-2 interleukine), kinine, eikosanoid, thrombocyte activation factor and nitrogen monoxide (NO) that intensify inflammation reaction.

Traditional treating of sepsis and complications present in antibiotic therapy, improving of tissue perfusion, preserving chemodynamic parameters and organ functions, removing the source of sepsis by surgical or other intervention, did not cause a drop of mortality rate that still ranges from 30-90%.

Since the organism dysfunction are too strong and uncontrolled inflammation reaction due to the invasion of microorganisms, immunomodulation reduces immunflammation response and prevents tissue dysfunctions. Potential biotherapy may reduce the consequences on the organism of the affected by interruption of one or more spots of molecular and cellular activities. For developing of new approaches in solving the problem of sepsis and treatment it is necessary to perform qualitative and quantitative investigations of sepsis that can be applied on animals (BOOKE et al. 1995, BURGMANN, 1995).

Treating of affected by sepsis
Application of antibiotics

Antibiotics are the main therapy of sepsis, and their application must be rational, based on microbiological diagnosis and sensitivity test. Until bacteriological results are ready, therapy must start with a combination of antibiotics that will provide effective covering of the broadest spectrum of causes. When deciding about the antibiotics, the one that are less toxic, good penetrating,
cheaper and more sensitive to bacteria is always chosen. Combination of aminoglycoside (gentamycin, amikacin) with penicillins (penicillin, ampycillin and cephalospores) are suitable.

It is considered that antibiotics necessary in treating of sepsis, paradoxically, may cause septic changes releasing endotoxin. It was proved that only polymixin group of antibiotics (polymixin-B, colistin and tirocilline) has antiendotoxin effect. Namely, polymix with entodoxin through ion and hydrophil, form a complex, binding with lipid A. Also, they may inhibit activation of complements in classic and alternative ways (CHOLLEY, 1995). Besides the above mentioned application of antibiotics, time for application and doses influences on quality of treatment. The patients without adequate antibiotic therapy have 10 to 15% higher mortality than the treated ones (COLLETTI, 1993).

Besides the antibiotics, application of corticosteroids is usual for inflammation reaction with those with sepsis or septic shock. In some clinical investigation the efficiency of cortico preparations was proved, while in some investigations negative reports are given. It is known that corticosteroids have many positive effects in inflammation process and are reflected in prevention of complement activation, neutrophil aggregation, slow biding of complements with antibodies, stopping metabolite arachidon acid synthesis by inducing synthesis of lipomoduline, stopping nitrogen-monoxide (NO), platelet activation factor (PAF) and preventing release of TNF and interleukin-1 (IL-1). However, protective mechanism of corticosteroids is still unexplained. It is known that glucorticoids, contrary to endotoxin (lipopolycharid, LPS), stimulate neoglucogenesis, so the protective mechanism could also be bind with this process. There are also opposite opinions on therapeutic effects of glucocorticosteroid, since they are introduced in the organism through pharmacological doses, so mechanisms of its affect are still unexplained. Their application weakens protection of cells and organism from the system of maintaining homeostasis, activated by heat stress and stress of acute sepsis phases. Int the models with animals, corticosteroids show favorable affect only if they are applied early. So, for example, if white mice are injected with LPS, 91% of animals dies, but if they are simultaneously injected with LPS containing cortisone, 80% of animals die. If LPS is given 4 hours before cortizone, then, in the same experimental conditions, the rate of surviving is only 16% (????, 1985). Some clinical experience, although only few, show that this may not be the case with humans, i.e. there are good reasons to give corticosteroids even in late phases of sepsis. Yet, treatment with corticosteroids should be started as soon as possible and stopped after two to three days (?????, 1994).

Lately, the role of unsteroid anti-inflammatory drugs have gained considerable interest in treating of sepsis (ÖINARELLO, 1993). Effect of eikosanoid-metabolite of arachidonic acid, that are known as leukotrienes and postglandline, in pathogen mechanism of sepsis, adult respiratory distress syndrome (ARDS) and multiple organ disfunction (MODS), are proved by findings of higher concentration of enzyme phospholipase A2 (PLA2) in the cases of humans, but also in the models with animals. Since that release of
arachidonic acid happens, in great extend, through phospholipase enzyme A2. Preventing, i.e. weakening of this enzyme, leads to reducing the intensity of organ disfunctions caused by inflammation, as well as in improving the outcome of sepsis. Methyl-prednisolone and dexametazon are considered the most important inhibitors of enzyme PLA2. In the sepsis model in animals, administration of inhibitor PLA2 result in reduced mortality of experimental animals from 80 to 40% (DiNARELLO, 1994).

Among the most important mediator/modulator of arachidonic acid metabolite in development of sepsis thromboxane A2 (Tx), prostanglandine (PG) and leukotrienes are included. Administration of methyl-prednisolone significantly reduces the content of all the investigated eicosanoids, but indomethacin only reduces concentration of prostaglandin, however not leukotriens in liver of the experimental animals. Both mentioned drugs improve survival of rats in septic shock. Less success of prostaglandins synthesis inhibitors (experimental animals in sepsis, i.e. on inhibitor of phospholipase A2 (methyl-prednisolone) is explained by emphasizing the effect of peptidoleukotrine as well by shortening protective effect of prostaglandins by indomethacin. There are numerous investigations on advantages in therapy by inhibitor enzyme cyclo-oxgenase during sepsis. In previous research, among the others, ibuprofen, indomethacin, naproxen and meclofenamate have been examined. The above mentioned cyclo-oxgenase inhibitors increase surviving, reduce dysfunctions of the lungs and improve hemodynamic features of experimental animals affected by sepsis (DORNUBUSCH, 19%, EVANS, 1993). It is proved that, out of the products of metabtic destruction of arachidonic acids through lypo-oxgenase enzyme, for the course and for the outcome of sepsis, the most important are leukotrienes (LT), first of all peptidoleukotrine (LTC5, LTO4, LTE4). When injected into experimental animals, they cause state similar to shock, miocardial depression and large plasma extravazacition. In pathogenetic mechanism of sepsis, peptidoleucotriene plays an important role, what is proved by the facts that by using antagonist receptor, as inhibitors of leukotrienes synthesis, it successfully prevents development of experimental edotoxin shock (FREUDENBERG, 1993). There are also data that point on large systemic production of peptidoleukotrine after injecting of endotoxin in vivo. Tromboxan ?? (????) plays the most important role in developing of pathological disorders, first of all lung hypertension, reduced lung flexibility, as well as trombocytopenia in sepsis, from cyclo-oxgenase products of arachidonic acid. It was proved that antagonists of receptor TXA2 improve chemodynamics, chematologic parameters, as well as survival of the experimental animals exposed to septic shock (GAAR, 1994).

Other possibilities of protection of organism affected by sepsis

Insufficient knowledge on sepsis pathogenesis caused difficulties and confusions in pharmaceutical therapy, because it was often limited to chemodynamic disturbances that were most visible and accessible for measuring. It is considered that in the mechanism of all septic processes, a reduced supply of
tissues by oxygen is present, and that depends on quantity of transported oxygen, 
minute heart capacity and possibilities of exploitation of oxygen. Application of 
sympathomimetic amines, first of all catecholamine that expresses its 
pharmacological effects through three kinds of receptors (alpha, beta and gamma),
and should provide optimal heart capacity and thus ensure correct tissue perfusion.
Adrenaline and noradrenaline have stimulating effect on alpha and beta-receptors,
isoprenaline and dobutamine on beta receptor. Dopamine equally stimulates all 
three kinds of and thus enlarges minute heart capacity, but also consumption of 
oxygen, what is hardly bearable (GALANOS., 1995). Besides ethyological therapy, 
in treating of sepsis beside symphomimetic amines, it is obligatory to correct 
hypovolemic, acid-base and electrolytic disturbances (GoYERT, 1995).

In many investigations the role of endogenous opioid system was proved, 
first of all beta-endorphin in pathogenesis of sepsis. Intensive release of 
endogenous opioid in early phase of sepsis causes myocardial depression, reduces 
mean artery pressure and flow of blood through heart. Positive results were 
obtained by application of antagonists for opioid receptors, out of which naloxone 
is the best known. Since naloxone blocks all opioid receptors only in larger doses, 
it can be assumed that positive effects may be expected only when larger doses are 
temporary given, what contributes preserving better condition of cardiovascular 
system and gaining on time for application of causal therapy (antibiotics, surgical 
intervention). After many investigations of naloxone, the results of survival of the 
affected by sepsis and septic shock were not satisfactory (UACKSHAW, 1990).

Clinical investigation proved that unbalance plays the central role in 
pathological processes of sepsis, that also includes neutophile activation. It is a 
consequence of differences caused by oxidative and antioxidative activities. 
Measuring of acidum ascorbicum (vitamin C) and tocopherolum (vitamin E) is an 
indirect method for detection of increased production of free oxygen radical. It is 
known that vitamin C is the first cleaner that enters into bloodstream and, together 
with vitamin E, prevents dysfunction of cells by lipid peroxidation. Therapy by 
antioxidant remedies in clinical sense may be defined as a process that moderates 
or stops dysfunctions on the tissues cause by oxydans. Therefore, early 
compensation of micronutritive remedies (vitamin C, vitamin E, beta carotin, zinc, 
manganese, copper, iron and selenium) in severe cases of sepsis may help 
protection of the organism.

Supportive therapy, besides other things, means also application of 
unspecific opsonin and cryoprecipitate in prophylaxis and treating of sepsis, 
because they improve elimination of infective agents, disintegrating products and 
detritus from circulation (HEIDEL, 1989).

New approach in treating of the affected by sepsis 
Neutralization of endotoxin

Intravenous or intraperitoneal injection of endotoxin causes dramatic 
changes in the organism of the experimental animals, because toxicity belongs to 
lipid A, as biological active component of endotoxin (KNAUS, 1996). Endotoxin
interacts with lipoproteins of high density, albumines, immunoglobulines, C3 complement component and binding protein. Endotoxin binds with monocyte through molecule CD12 and other receptors. In interaction macrophage and endotoxin molecule family CDn/CDis is included, as well as two proteins with molecular mass 55 and 65 kDa. Endotoxin binds and bactericid protein, that increases permeability, and if sound in azurophil granules of neutrophil. Application of antibodies, specific for endotoxin, significantly reduces toxic effect of endotoxin on experimental model of laboratory animals and in volunteers that were vaccinated polyclone (J5) mutant E. coli. So far mice of endotoxin activity. Application of monocline antibodies towards endotoxin significantly increased the percentage of surviving, but in over 50% cases toward them antibodies were developed. However, human monocline antibodies class IgM showed safe, but less immunogene. Preliminary investigation of the affected from sepsis with antipolysacharid and IgG antibodies contribute reducing mortality rate from 80 to 50%. Namely, application of IgG antitoxicin antibodies reduces endotoxin concentration in plasma of those affected by sepsis, and thus contribute to more successful effect of this immunotherapy in latter phases of treatment. According to some authors, antitoxin antibodies that were directed towards lipid, did not have positive effect on surviving of the affected. There are numerous questions like indication, efficiency, harmfulness, questions concerning prices, what is very complex. Beside antibodies, natural or recombined antagonist protein, that achieve bactericide effect by increasing of permeability and stopping toxic activity of endotoxin (MOLLOY, 1993).

Application of inhibitors of cytokine activity

Inflammatory and immune process during sepsis and septic shock consists of complex biological cascades that can be weakened by new anticitokine biotherapy. First of all, it is thought on TNF-OC or IL-1. It is still not clear which moment in the series of events during sepsis and septic shock is control moment where stopping could revert the process on previous better condition (NOMURA, 1993). TNF and IL-1 regulate proinflammatory, protective immune response on infection and the defense of the host in sepsis: to activate neutrophile, macrophage and lymphocyte, intensify expression of gene and release proteins of acute inflammatory phase and stimulative factor of granulocyte colonies (SFGK), induce antiinflammatory cytokine IL-4, IL-6 and IL-10, IL-Ira and transforming growth factor b, reducing the level of TNF or IL-receptors (PITTNER, 1993).

More knowledge on structure of cytokine inhibitor activity proved information that they are soluble receptors, antagonists and inhibitors of cytokine synthesis (PUTTERMAN, 1989). These substances are especially useful from the point of clinical application. As natural component in their application, formation of human antibodies appear in mice monocline are avoided that appear towards mice monocline antibodies, specific for lipid of A endotoxin. Soluble receptors bind them in circulation, and thus initiate effective cell functions. Receptors for TNF-cc are isolated, cloned and produced in recombined way. When
applied to mice one hour before application of TNF, they protect from toxic effect of this cytokine. Also, animal model of sepsis application of large doses of recombined antagonist OLI (IL-Ira) neutralizes many harmful effects (SIEGEL, 1991). Preliminary results of its affect on human show that in majority of the affected with sepsis this therapy may be effective, although the effect on human are still not harmonized.

The main obstacle in application of monoclon antibodies, first of all TNF, again cytokine is their fast release. Therefore optimal time for application of antibodies usually passes. The majority of antibodies, applied against cytokine, are of mice origin, and against them humans produce antibodies. The main obstacle why it is not used in practice is its high price.

Nitrogen monoxide - on of the target in sepsis therapy Nitrogen monoxide (NO), as a gas of low molecule mass, easily goes through cell membrane, functions as a neurotransmitter, regulates tonus of blood vessel, inhibits aggregation and thrombocyte adhesion. In concentration, that is higher than those necessary for intercellular communication, it expresses antitumor and antimicrobe activity. In physiological conditions it is produced in endothelium by chemical action of NO synthetase (NO-s) depending on calcium and calmoduline. Production of NO is controlled by vasodutation acetylholine and histamine. Inflammatory mediator induce in endothelium cells and in smooth muscles of blood vessels, induced form of NO synthetase (NO-s) that is independent from calcium. Different from physiological NO-s, the induced one is not under control of usual regulatory mechanism, accordingly in an uncontrolled way enlarges production of NO in the affected from sepsis (TANEYAMA, 1989). Enlarged production of NO during sepsis is followed by numerous harmful effects as hypotension, myocardial depression, citotoxic effect, tissue dysfunctions and weakening the organs. Inhibition of NO production presents important, new approach in treating oh hypotension in sepsis. Based on the first favorable results in application of NO-synthesis (? -nitro-L-arginin methyl ester-L-NAME and No-mono methyl-L-arginine-L-NMME) hypotension, a new approach was used in clinical investigation of affected by sepsis and those with cancer where cytokine therapy was used (YELICH, 1990). Clinical investigations, that were carried out, were followed by numerous harmful effects, i.e. with expected inhibition of induced NO-synthetase, they inhibited constitutative NO-synthetase, and thus the affected from sepsis was deprived from necessary protective effect of NO. The solution is searched in finding selective NO-synthetasis inhibitor directed exclusively on induced form of NO-synthetasis and form for influence on definite vascular segment.
CONCLUSION

Improvement in understanding of pathogenesis of sepsis provides condition for developing a strategy with the help of immunomodulators. In that sense, new research therapy protocols is created with the aim to discover the starting factors at the beginning of sepsis, increase defense of the host, prevent interaction of leukocyte with endothelium, inhibit citokine, vazoactive substances and lipid mediators. No new approach in treating of sepsis and septic shock proved to be efficient and safe. The experience showed that in using inflammatory agents in sepsis and septic shock is more complicated than it was thought at the beginning. Individual positive results in the experiments with animal could not be applied in clinical investigation of the affected.

Future therapeutic approach in treatment of sepsis will depend on selection of mediatory inflammatory reaction which is inhibited or intensified, and on the choice of right moment. There is a need for new laboratory and mediatory tests that can quickly measure level of specific bacterial toxins or inflammation reaction in the host in order to treat the affected. Therefore, in last years possibility of therapy application is directed toward time of recovery from immunosuppression that is present in those affected by sepsis.

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Sepsa kao sistematska inflamatorna reakcija organizma i dalje predstavlja veliki terapijski problem. Reanimaciona i potporna terapija zavisi od stadijuma u kojem je holest prepoznata kao dalji nacin lecenja, mogu uticati na smanjenje mortaliteta koji se kreće od 30 do 90%.

Sam pristup u ublažavanju posledica štetnih delovanja septickog stanja vrlo je kompleksan. Pored običajnih terapijskih mera, koje obuhvataju primenu antibiotika, antišok terapije, antiinflamatornih lekova, antagonista endomorfinskog sistema i prostaglandina, zadovoljavajući rezultati se dobijaju i primenom antiendotoksinskih antitela, antimonokina, antikomplementnih antitela antioksidantnih sredstava.

Istraživanja novih lekova zasnivaju se na principima modifikovanja i zaustavljanja nepoželjnih metabolickih, odnosno biohemijskih i receptorskih aktivnosti celija. Upotreba ovih lekova našla je primenu u eksperimentalnim studijama u kojima se postiže dobri rezultati. Navedeni noviji lekovi nalaze svoje mesto i u kliničkoj praksi a njihova pravovremena primena garantuje uspešnu terapiju.

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