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Cytoprotection by omega-3 fatty acids as a therapeutic drug vehicle when combined with nephrotoxic drugs in an intravenous emulsion: Effects on intraglomerular mesangial cells

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ABSTRACT

During therapeutic interventions, blood concentrations of intravenously applied drugs are higher, and their onset of pharmacological action is faster than with other routes of drug administration. However, acute drug therapy often produces nephrotoxic side effects, as commonly seen after treatment with Ketorolac or Gentamicin leading to questions about their use, especially for patients at risk for acute renal failure. Omega-6(n-6) and omega-3(n-3) polyunsaturated fatty acids (PUFA) affect eicosanoid metabolism, which plays a role in the regulation of inflammation. Eicosanoids derived from n-6 FA have proinflammatory and immunoactive functions, whereas eicosanoids derived from n-3 PUFA have anti-inflammatory and cytoprotective properties. We hypothesized that providing such injectable drugs with nephrotoxic potential in combination with n3-PUFAs from the outset, might afford rapid cytoprotection of renal cells, given the recent evidence that intravenously administered n3-PUFAs are rapidly incorporated into cell membranes. We used intraglomerular mesangial cells (MES13) that are sensitive to treatment with Ketorolac or Gentamicin instead of proximal tubular cells which do not respond to Ketorolac. We found a significant inhibition of Ketorolac (0.25, 0.5, 1 mM) or Gentamicin (2.5, 5 mM) induced cytotoxicity after pretreatment of MES13 cells with 0.01% of 20%w/v LipOmega-3 Emulsion 9/1, containing 90:10 wt/wt mixture of fish oil derived triglycerides to medium chain triglycerides.

1. Introduction

The underlying toxicity of many drugs to vital organs of the body is often a result of localized ischemia which triggers a series of complex biochemical events related to hypoxia, inflammation, and with the potential for subsequent oxidative stress with reperfusion, all of which

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induce cellular damage. This is particularly true in the critically ill patient in the intensive care unit with an ineffective circulating blood volume (e.g., mean arterial blood pressure < 60 mmHg) causing moderate to severe hypoperfusion to vital organs such as the kidney, producing acute tubular necrosis. In this setting, the likelihood or severity of acute kidney injury (AKI) can be aggravated by the concurrent administration of injectable nephrotoxic drugs, with mortality rates from acute renal failure ranging from 25 to 64% [21].

Thus, the combined impact of ischemic insult to aerobic tissues, from both disease(s) and drug(s), greatly accentuates the potential for damage to vital organs in the critically ill. During ischemia, oxygen, as well as other vital nutrients become limiting to dependent tissues such as the kidneys, causing mitochondrial dysfunction [4]. Blood flow to the kidneys as a fraction of the total cardiac output is approximately 20% [11]. Thus, even limited reductions in normal blood flow to the kidneys resulting from a compromised circulating blood volume as described above, can have devastating consequences for renal tissues, especially during the concomitant intravenous administration of nephrotoxic drugs in full therapeutic doses.

Polyunsaturated omega-3 or n-3 fatty acids (n3-PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from fish oil triglycerides, have been shown to mitigate the nephrotoxic effects of cyclosporine A in an experimental animal model [9]. Animals were pre-fed via gastric lavage for 14 days prior to nephrotoxic drug exposure in order to obtain sufficient incorporation of n3-PUFAs into plasma cell membranes to exert their cytoprotective effects. Although this approach would seem viable in patients receiving such therapy in the long-term setting (e.g., lifelong immunosuppression with cyclosporine A for organ transplantation), the requisite time course for pre-treatment with n3-PUFAs in the acute care setting in patients with, for example, life-threatening blood infections, or, for acute postoperative pain, is not achievable. That is, acute drug therapy is often vital with a potential nephrotoxin given intravenously to achieve higher concentrations more rapidly and completely than achievable with oral administration in order to improve therapeutic efficacy, which also increases the potential for adverse renal effects that are generally related to drug levels. However, we hypothesize that providing such injectable drugs in combination with n3-PUFAs from the outset, may afford rapid cytoprotection of renal cells, especially in light of recent evidence that intravenously administered n3-PUFAs are rapidly incorporated into plasma cell membranes within 1 h of infusion after a single dose [6]. Once incorporated into cell membranes, a key step to exert cytoprotection, n3-FAs dramatically modulate the body's response to inflammation, oxidative stress, ischemia and immune function through several downstream bioactive mediators, (e.g., cytokines, prostaglandins, thromboxanes, leukotrienes, resolvins, protectins, etc.) [2].

The purpose of this investigation was to study the potential cytoprotective effects of omega-3 fatty acids against known nephrotoxic drugs in an in vitro murine intraglomerular mesangial cell model to establish proof of concept. The combination of "cytoprotective excipients" with selected nephrotoxic drugs constitutes a unique opportunity for potentially safer intravenous therapy in the acute care setting, where the inclusion of omega-3 fatty acids could also serve as a secondary supportive, but important, active pharmaceutical ingredient, to form a novel *therapeutic* drug vehicle.

2. Material and methods

At the outset, SV 40-transformed mouse mesangial cells, MES 13 (CRL-1927^{TM}; purchased from the American Type Culture Collection [ATCC-LGC Standards GmbH], Wesel, Germany) were used.

The selection of these cells, as opposed to proximal tubule cells typically associated with renal tubular damage, was based on observations that nephrotoxicity from drugs such as Gentamicin is related to their direct effect (proliferation and apoptosis) on cultured mesangial cells [20] In contrast to MES13, the HK-2, an immortalized proximal tubule epithelial cell line from normal adult human kidney have not shown any response after treatment with Ketorolac (not shown). Thus, we chose to study MES13 and two nephrotoxins typically given by intravenous administration in the ICU setting: The potent analgesic, Ketorolac, and the aminoglycoside antibiotic, Gentamicin.

2.1. Cell lines and culture conditions

The MES 13 cells were cultured in ATCC complete growth medium: the basic medium for these cell lines is a 3:1 mixture of ATCC-formulated Dulbecco's Modified Eagle's Medium [ATCC (DMEM)], and Ham's F12 medium (PAA Laboratories GmbH, Cölbe, Germany) with 14 mM HEPES, supplemented with 5% fetal bovine serum (FBS), 100 U/ml penicillin, 0.1 mg/ml streptomycin. Under these culture conditions the MES 13 retained many of the differentiated characteristics of mesangial cells. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air; the medium was changed every 48 h.

2.2. Substances under test

2.2.1. The control emulsions

20%$_{w/v}$ Lipofundin N, containing 20% soybean oil only triglycerides, Lot No 111258082.

2.2.2. The test emulsions

20%$_{w/v}$ LipOmega-3 Emulsion 5/5, containing 50:50$_{wt/wt}$ mixtures of fish oil (FO) derived triglycerides and (MCTs), solution number 356. Lot No 11182049, 20%$_{w/v}$ LipOmega-3 Emulsion 7/3, containing 70:30$_{wt/wt}$ mixtures of fish oil (FO) derived triglycerides and medium chain triglycerides (MCTs), respectively, solution number 358. Lot No 11182050; 20%$_{w/v}$ LipOmega-3 Emulsion 9/1, containing 90:10$_{wt/wt}$ mixtures of fish oil (FO) derived triglycerides and medium chain triglycerides (MCTs), respectively, solution number 359. Lot No 11202049. All lipid emulsions by B. Braun Melsungen AG, Melsungen, Germany.

The fish oil used in all 3 fish oil-containing emulsions tested is highly enriched, and we have data on this oil from