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Serum microRNAs-217 and −375 as biomarkers of acute pancreatic injury in rats

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ABSTRACT

Pancreatic injury in rats is primarily detected through histopathological changes and conventional serum biomarkers such as amylase and lipase. However, amylase and lipase have a short half-life and are markers of acinar, not islet cell injury. We investigated whether circulating microRNA (miR) levels that are enriched in acinar cells (miR-217, miR-216a/b) or islet cells (miR-375) could serve as markers of pancreatic injury. Rats were treated with a single dose of either vehicle, streptozotocin (STZ), caerulein, or acetaminophen (APAP), and necropsied at 4, 24, and 48 h. Pancreas, liver, heart, kidney and skeletal muscle were analyzed for histopathology. Blood was collected at necropsy and processed to serum for amylase/lipase enzymatic determinations and miR qPCR analysis. Caerulein induced degeneration/necrosis of acinar cells at 4 h that persisted for 48 h. Caerulein-induced injury was associated with increases in serum amylase/lipase (4 h), miR-216a/b (4, 24 h). In contrast, serum miR-217 was detected at all time points examined. STZ did not induce increases in either amylase or lipase but did induce increases in miR-375 levels at 4 and 24 h. No increases in miR-375 were observed in caerulein-treated rats, and no increases were observed in miR-217 and miR-216a/b in STZ-treated rats. APAP induced centrilobular necrosis in the liver 24 h after treatment, but did not induce pancreatic injury or increases in miR-217 or miR-375. Our results suggest that miR-217 and miR-375 represent promising biomarkers of pancreatic injury in rats.

1. Introduction

Pancreatitis is a serious, potentially fatal disease with a varying severity of presentation (Meher et al., 2015). Two enzymes derived from pancreatic acinar cells, amylase and lipase, in combination or individually, remain the most widely used biomarkers for clinical diagnostics of acute pancreatitis. However, there are several limitations with the use of amylase and lipase as biomarkers of pancreatic injury in humans and in experimental animals. The half-life of serum amylase and lipase is short in duration. Studies suggest half-life of amylase and lipase in humans is about 7–18 h (Junge et al., 1985) and between 1 and 2 h in rats (Papp et al., 1982). Therefore, increases in serum pancreatic enzymes may not be observed if sufficient time elapses between the onset of acute pancreatic injury and the time blood is collected for evaluating amylase and lipase activities.

Another limitation of the use of the pancreatic biomarkers, amylase and lipase, involves their lack of specificity for pancreatic injury. Amylase and lipase can be released into the serum from several tissues beside the pancreas (Usborne et al., 2014). As such, serum lipase and amylase can be increased in conditions other than pancreatitis such as nonmalignant hepatobiliary and gastrointestinal diseases, sepsis, renal and pulmonary failure (Diani et al., 1998).

Decreases in ß-cell mass in pancreatic islets are associated with the development of diabetes in humans. However, there is currently no established circulating biomarker for measuring injury to pancreatic ß-cells. Insulin is secreted by ß-cells which is involved in glycemic control and therefore it may be possible to assess pancreatic ß-cell mass by measuring circulating levels of insulin. However, there are major problems with this approach. First, there is a considerable functional reserve in insulin production by ß-cells. Therefore, decreases in circulating insulin levels would only be expected to occur once severe damage to

Abbreviations: APAP, acetaminophen; Ct, cycle threshold; DPBS, Dulbecco's Phosphate-Buffered Saline; ip, intraperitoneal; miR, microRNA; po, oral gavage; STZ, streptozotocin.

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pancreatic ß-cells occurs. It has been demonstrated that a 30–50% decrease in ß-cell mass is required to produce only mild changes in blood glucose and insulin levels in humans and experimental animals (Kendall et al., 1990; Masiello et al., 1998) thereby limiting the sensitivity of these parameters for detecting ß-cell injury. Second, the half-life of insulin is extremely short (Rubenstein et al., 1972) which can make measurement of peak circulating insulin levels difficult.

In recent years, microRNA (miRs) have been evaluated as potential biomarkers of tissue injury. MiRs are endogenous, small (21–22 nucleotides), single-stranded, noncoding RNAs that regulate gene expression at the post-transcriptional level (Bartel, 2004). Many miRs are highly conserved across many species, and are expressed in a tissue/organ-specific manner. MiRs have been demonstrated to circulate in a highly stable, cell-free form in body fluids, including serum (Ambros 2004; Chen et al., 2008; Gilad et al., 2008; Lawrie et al., 2008), plasma (Mitchell et al., 2008), saliva (Park et al., 2009) and urine (Hanke et al., 2010). These characteristics make miRs excellent candidates for biomarkers of target organ toxicity.

Sources of such circulating extracellular miRs can include the following: (i) tissue injury with passive leakage from broken cells due to chronic inflammation, cell apoptosis or necrosis. (ii) turnover of normal cells especially cells with short half-lives such as platelets. (iii) active secretion of miRs in microvesicles, including exosomes and shedding vesicles. Microvesicles are membrane enclosed cell fragments released by almost all cell types under both normal and pathological conditions (Cocucci et al., 2009; Mathivanan et al., 2010; Ratajczak et al., 2006; Simons and Raposo 2009; Thery et al., 2002). In the case of target organ toxicity, passive leakage of miRs due to tissue injury would be expected to be a major mechanism for increases in circulating miRs.

Recently, several studies have evaluated miR-375, miR-216a/b, and miR-217 as potential markers of pancreatic injury in rodents (Endo et al., 2013; Erener et al., 2013; Goodwin et al., 2014; Kong et al., 2010; Usborne et al., 2014). Studies have demonstrated that miR-375 is highly expressed in pancreatic ß-cells in humans (van de Bunt et al., 2013), mice (Poy et al., 2009) and islet cells in the rat (Bravo-Egana et al., 2008), while miR-216a/b and miR-217 are enriched in the acinar cells of pancreas in humans and in rats (Endo et al., 2013; Goodwin et al., 2014).

In the current study, rats were treated with a pancreatic islet ß-cell specific toxicant, (streptozotocin [STZ]), and a pancreatic acinar cell-specific toxicant (caerulein) and changes in serum miRs were profiled. The mechanism of islet ß-cell toxicity of the glucose analog, STZ, is not fully understood, but STZ is known to be preferentially taken up by ß-cells. Once STZ is taken up, evidence suggests that STZ alkylates DNA leading to sequence of events eventually leading to cell death (Bennett and Pegg, 1981). Caerulein is an oligopeptide that is hypothesized to exert its toxicity through the induction of oxidative stress in acinar cells. According to this hypothesis, oxidative stress damages intracellular membranes releasing digestive enzymes leading to death of acinar cells (Arafa et al., 2009). In addition, rats were treated with the hepatotoxicant, APAP, to determine the relative specificity of candidate miRs for pancreatic injury.

The objectives of the study were twofold. First, to identify miRs that are potential biomarkers of acute pancreatic injury in rats and compare them with conventional biomarkers of pancreatic injury, and secondly, to determine if specific miRs could be used to differentiate between acinar and ß-cell pancreatic injury.

2. Materials and methods

2.1. Chemicals

STZ, APAP, caerulein and sodium citrate were purchased from Sigma Aldrich (St. Louis, MO, USA). DPBS was purchased from Lonza (Walkersville, MD, USA) and Methocel A4 M was purchased from Dow (Plainview, NY, USA).

2.2. Animals

All experiments were conducted with the approval of the Bristol-Myers Squibb Institutional Animal Care and Use Committee in an AAALAC-accredited facility. Male Sprague Dawley rats (Crl: CD) (12 weeks of age) were purchased from Charles River Laboratories, Kingston, New York, USA. Upon receipt, the rats were group housed in appropriate sized solid-bottom cages for 1 week prior to dosing. The rats were housed in a humidity and temperature-controlled room (targeted daily mean range: 30–70% RH; 68–79 °F) on a 12-h light/12-h dark cycle and provided Harlan Rodent Diet #2018C (certified 18% protein) and purified, chlorinated tap water ad libitum. Animals were observed at least once daily for changes in condition or behavior.

2.3. Experimental design

Fifty four (54) male rats were assigned to each of 3 toxicant-treated groups containing 12 rats each and 2 vehicle control group containing 9 rats each (Table 1). The fewest number of animals per group were used to accomplish the objectives of the experiment and to allow for appropriate statistical methods. Doses of STZ (150 mg/kg), caerulein (0.05 mg/kg) and APAP (1500 mg/kg) were selected based on the results of published reports (Goodwin et al., 2014; Heinloth et al., 2004; Schulingkamp et al., 2005). All

Table 1
Experimental Design.

Compounds	Mechanism of Action, (Target Tissue Toxicity)	Rats per group/Rats per time point	Time points (h)[a]	Dose; vehicle; route of administration (dose volume)
Vehicle Control	–	9/3	4, 24, 48	0.1 M sodium citrate buffer (pH 4.5); ip; (2 mL/kg)
Vehicle Control	–	9/3	4, 24, 48	Methocel A4 M; po; (10 mL/kg)
Streptozotocin	Cytotoxic glucose analog that induces DNA alkylation (Pancreatic β-cells)	12/4	4, 24, 48	150 mg/kg; 0.1 M sodium citrate buffer (pH 4.5); ip; (2 mL/kg)
Caerulein	Oligopeptide that induces oxidative stress and release of digestive enzymes (Pancreatic Acinar cells)	12/4	4, 24, 48	0.05 mg/kg; DPBS; ip; (2 mL/kg)
Acetaminophen	Analgesic, Negative Control (Liver)	12/4	4, 24, 48	1500 mg/kg; Methocel A4 M; po; (10 mL/kg)

[a] Rats were administered a single-dose of compound or vehicle then necropsied at either 4, 24 and 48 h after administration. Abbrevations: ip, intrapertioneal injection; po, oral gavage.