MANUSCRIPT

An observational study to justify and plan a future phase III randomized controlled trial of metformin in improving overall survival in patients with inoperable pancreatic cancer without liver metastases.

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Abstract

Purpose

Metformin has plausible direct and indirect anti-cancer properties against pancreatic adenocarcinoma cells. However, metformin may only be efficacious in patients with inoperable pancreatic ductal adenocarcinoma (PDAC) without liver metastases. Absorption may be decreased by gastrointestinal symptoms and proton pump inhibitors (PPIs). We aimed to justify and inform a future phase III trial of metformin versus placebo on survival in inoperable PDAC by documenting prevalence of patients meeting eligibility criteria, gastrointestinal symptoms and PPI use.

Methods

Patient notes with PDAC were reviewed at a large teaching hospital over a two-years. Study variables were obtained from multiple sources of information.

Results

141 participants were identified (51.8% female), of which 37.6% were not prescribed metformin at diagnosis and had no radiological hepatic metastases. Characteristics were similar between non-metformin and metformin users. In eligible patients, 65.2% reported nausea and vomiting and 46.2% prescribed PPIs.

Conclusion

Approximately a third of all patients with inoperable PDAC are eligible for a future trial of metformin, allowing an estimate of the number of hospitals required for recruitment. Nausea and vomiting are common and should be managed effectively to prevent trial dropouts. PPI use is frequent and their influence on metformin's pharmacodynamic actions needs to be clarified.

Keywords

Pancreatic cancer, metformin, trial eligibility, drug absorption.

Introduction

New adjuvant chemotherapies are urgently required to treat patients with inoperable pancreatic adenocarcinoma (PDAC), as the survival is extremely poor.(Ferlay J 2013.) The current treatment options are chemotherapy with either gemcitabine or FOLFIRINOX, although the proportions of patients surviving one year are still only 20% and 50% respectively.(Conroy et al. 2016; Conroy et al. 2011) The oral hypoglycemic drug, metformin, may be a new potential therapeutic option in inoperable patients without liver metastases, as firstly it has direct anti-cancer properties against PDAC cells as demonstrated in laboratory experiments.(Wang et al. 2008; Zhou et al. 2016) Secondly, metformin may have indirect anticancer actions by inhibiting gluconeogenesis in the liver, with consequent lower blood glucose levels decreasing the secretion of excess potentially mitogenic insulin which promotes pancreatic carcinogenesis.(Sasaoka et al. 1994)

To date, metformin and its effect on overall survival has been investigated in PDAC in two randomized controlled trials (RCTs).(Ding et al. 2014; Reni et al. 2016) Both of these reported null findings but several study limitations could explain these results. In the first from the Netherlands, (hazard ratio (HR) 1·06 (95% CI 0·72–1·55), p=0·78), participants in the metformin arm had higher baseline levels of the tumor marker, CA19-9, than in the placebo arm.(Kordes et al.) Therefore, more patients allocated metformin had cancers with greater progressive tendencies than participants who received placebo. Furthermore, more patients in the active arm discontinued metformin due to drug side effects, such as nausea and vomiting (22% vs 13%, p=0.21). Both these reasons may have masked any potential therapeutic benefit of metformin in the intention-to-treat analysis. The second trial was an open-label randomized phase II study of 60 patients conducted in Italy.(Reni et al. 2016)

Here only 77% of the required 78 patients were recruited, and a null effect was reported (HR 1.56, (95% CI 0.87-2.8), p=0.13). Therefore, in future, larger samples sizes are needed to ensure effect sizes can be detected, there are similar baseline cancer stages between allocation arms, and clinical measures are incorporated to ensure tolerance with the study drug.

There are plausible biological reasons why metformin may work better in patients with locally advanced PDAC, without liver metastases.(Broadhurst and Hart 2018) The progression of the primary pancreatic cancer itself may be impaired if metformin maximally inhibits hepatic gluconeogenesis and reduces excess mitogenic insulin release. This mechanism will be most effective on the pancreatic lesion if the liver is not affected by metastases, so gluconeogenesis can be maximally inhibited. Furthermore, if liver metastases are present, this mechanism is less relevant as the cancer has already metastasized there. Quantifying their prevalence in patients with inoperable PDAC is therefore required, to give an assessment of the generalizability of metformin's potential use in patients with inoperable PDAC. Additionally, the prevalence of PPI use needs to be investigated, as metformin's efficacy may be impaired by their concurrent prescription.(Kim et al. 2014) PPIs may inhibit organic cation transporters (OCTs) and impair metformin uptake in the liver. The latter is mediated by the membrane transporter OCT1, and to a lesser extent by OCT3.(Graham et al. 2011) The prevalence of symptoms, such as nausea and vomiting, which could reduce its absorption, needs to be documented.

The aims of this clinical observational study were to provide information to both justify and plan a future phase III randomized double-blind controlled trial of metformin versus placebo assessing overall survival in inoperable PDAC. We investigated, for the first time, the proportions of inoperable patients without liver

metastases and not prescribed metformin who would be eligible for such a trial, PPI use in this patient group and the frequency of gastrointestinal symptoms.

Methods

This study was a retrospective, cross-sectional clinical observational design which reviewed the notes of patients with inoperable PDAC treated at a large teaching hospital in Norfolk, United Kingdom. Patients were identified from the multidisciplinary team (MDT) meeting records, who were eligible if aged ≥18 years and diagnosed with PDAC between 1st January 2015 and 31st December 2016. For patients with no histocytological confirmation, study eligibility required the relevant clinical symptoms and radiological evidence of PDAC.

The study variables were recorded from the date of diagnosis till death or the censor date (31st March 2018). Variables were divided into i) those factors affecting participant *eligibility* into a future trial (not prescribed metformin, no hepatic metastases at diagnosis) and ii) those factors affecting *drug absorption* and *tolerance* (PPI use, nausea and vomiting, and steatorrhea). We documented the prevalence of renal disease and baseline estimated glomerular filtration rate (eGFR), as metformin is contraindicated in patients with severe kidney disease (eGFR less than 30). The data sources were: primary care letters, records of hospital consultations, radiology and histology reports.

In the analyses, continuous data were described with the mean (plus standard deviation) or median (plus interquartile range), according to the nature of their distributions. Categorical variables were reported as percentages. Continuous variables were compared between groups (metformin users and non-users) using the students t-test, non-continuous variables with the Mann-Whitney test, and proportions with the Chi-square test. SPSS software was used for analyses.

Ethical approval was granted by Cambridgeshire and Hertfordshire Research Ethics Committee (REC reference 18/EE/0114) and regulatory approval from the Health Research Authority (IRAS ID 238050).

Results

In total, 163 participants with potential PDAC were identified from the MDT records, during the two-year study period. Of these, 22 (13.5%) were excluded due to: operability (n=7, 4.3%), a non-pancreatic adenocarcinoma primary cancer e.g. cholangiocarcinoma (n=6, 3.7%) and neuroendocrine tumor (n=4, 2.5%), no formal PDAC diagnosis (n=3, 1.8%), date of diagnosis recorded in notes earlier than inclusion dates (n=1, 0.6%) and follow-up care outside Norwich (n=1, 0.6%). Therefore, there were 141 eligible participants with a median age at diagnosis of 70.0 years (range 47.0-96.0 years), of whom 51.8% were female (table I). The proportion with data which could not be ascertained for each variable ranged from 0%-32.5% (supplementary table I) including 2.1% for *hepatic metastases*, 2.1% for *DM status during follow-up*, 7.1% for *PPI use*, 5.7% *nausea and vomiting* and 32.5% for *baseline CA19-9*.

Eligibility for a future trial

Of the 141 participants, 21 (14.9%) were prescribed metformin at diagnosis and would therefore be ineligible for a future trial. There were no significant differences in demographic and lifestyle characteristics between metformin and non-metformin users (table I), although the performance status was of borderline significance (p=0.05). All clinical characteristics of the cancers were statistically similar between groups; although there were more advanced cancers in the non-metformin users of borderline significance (table II). Of the 120 non-metformin users, 65 (55.1%) had hepatic metastases who would be ineligible for a future trial. Therefore, 53 (37.6% of all) inoperable patients were not prescribed metformin at diagnosis and did not have liver metastases, who would meet full eligibility criteria. There were no significant

differences in demographics or tumor site in the pancreas between patients prescribed and not prescribed metformin without liver metastases (table III). In the 33 patients with DM at diagnosis (23.4%), including those prescribed dual hypoglycemic therapy, 63.6% were prescribed metformin, 42.4% gliclazide, 3.0% alogliptin and 12.1% insulin. During follow-up, 9 eligible participants (20.5%) developed new DM, with 2 of all participants (1.7%) prescribed metformin (table IV).

Factors affecting absorption and tolerance of the proposed investigational medicinal product (IMP)

PPI prescriptions were common, with 24 (46.2%) patients at diagnosis prescribed these drugs in the eligible group, which increased during follow-up to 33 (64.7%). H2 receptor antagonists were less commonly prescribed at diagnosis (7.5%) in those eligible, but again nearly doubled later. In eligible PDAC patients at diagnosis, nausea and vomiting were reported in 18 (34.0%), of whom most had no underlying identifiable cause on note review (table IV). This number nearly doubled during follow-up, of which 17.0% were due to duodenal obstruction, and all but one had a duodenal stent inserted.

Discussion

This study provides novel information to both justify and inform a future, phase III double-blind, parallel group, placebo controlled, randomized trial investigating if metformin improves overall survival in patients with inoperable PDAC without liver metastases. Overall, approximately a third of all patients with inoperable PDAC would meet the eligibility criteria. In the UK, there are approximately 3,000 patients per year who would benefit from metformin (if it is shown to be efficacious). At diagnosis, one in seven of all patients would be excluded as they were prescribed metformin, and in the remainder 55.1% had liver metastases so are ineligible. During follow-up, a further 20.5% developed DM, with a quarter of these subsequently requiring metformin. Patients may develop diabetes as a consequence of the cancer itself destroying β cells, and metformin is then the recommended first line treatment.(Chatterjee et al. 2017) In a trial, such patients would therefore need to stop the study drug, but are still followed up on an intention-to-treat basis or prescribed an alternative oral hypoglycemic drug. Consequently, for a per protocol analysis, the sample size would need to be increased slightly by 4% to mitigate against this loss. Importantly, the demographic and clinical characteristics of patients prescribed, and not prescribed metformin were similar.

We hypothesize metformin may work better in PDAC patients without hepatic metastases, if the drug's potential anti-cancer action includes inhibiting hepatic gluconeogenesis and reducing excess insulin secretion, which is mitogenic in the pancreas.(Sasaoka et al. 1994) The higher concentration of insulin in response to elevated glucose levels secondary to hepatic gluconeogenesis will be greatest at the site of the primary pancreatic cancer. Therefore, this mechanism of metformin is going to be potentially more effective on the primary pancreatic tumor, in patients

without liver metastases. In non-metformin users at diagnosis, a further 55.1% of all inoperable patients were ineligible for a future trial due to hepatic metastases. Therefore, in total 37.6% of all inoperable patients are eligible, which allows an estimate of the number of hospitals required to recruit a given sample size for a trial. Including PDAC patients with liver metastases may explain why a null effect was reported in the previous two trials.(Kordes et al. ; Reni et al. 2016) Ideally a threshold level of radiological hepatic metastatic involvement needs to be estimated, below which metformin may be therapeutic. In the CT reports reviewed, often hepatic metastases were described as 'multiple' without any quantification of their number or the proportion of the liver involved. If there are minimal and small metastases, adequate liver function may still be preserved. A validated and quantitative method for assessing the volume of hepatic metastases is needed to measure metastatic load. Unidimensional CT measurements, such as the diameter of metastatic lesions, are quick, simple to do, and are currently used in clinical reports. However, CT volumetric measurement is a more accurate method for assessing tumor burden at diagnosis.(Gonzalez-Guindalini et al. 2013; Suzuki et al. 2008) This is calculated by the radiologist tracing the outline of the lesions on each CT image slice.(Mantatzis et al. 2009) The area is then automatically calculated, with the volume computed by multiplying the area by slice width. The sum of slices equates to the total hepatic lesion volume. Volumetric measurement can be incorporated in the staging CT scan report, although it increases the reporting time by approximately 20 minutes.(Dello et al. 2011; Rothe et al. 2013) For a future trial, it is imperative that a threshold volume is decided for measuring hepatic metastatic volume, although currently this is unknown. The higher the cancer volume, the fewer patients would be eligible, although any threshold would be an estimate based on an assessment of clinical

plausibility. If a trial showed that metformin was efficacious, then those patients already prescribed metformin at diagnosis, but excluded from the trial would also benefit. The total proportion would be nearly half (45%) of all inoperable PDAC patients i.e. those without liver metastases.

The effectiveness of metformin will be influenced by its absorption, which would be reduced by symptoms including nausea and vomiting, and also prescription of other drugs which may impair its bioavailability. We documented nausea and vomiting were present in a third of eligible patients at diagnosis. Although, most causes of such symptoms were unknown, they may be due to the systemic effects of the malignancy itself. Of metformin users at diagnosis, most (85.7%) did not report nausea and/or vomiting, hence if these symptoms develop, they are unlikely to be due to the drug. Other factors which may impair drug absorption, namely duodenal obstruction and steatorrhea were less common, which can be addressed by duodenal stent insertion and the prescription of pancreatic enzyme replacements. The protocol of a future trial should contain a robust plan to manage gastrointestinal symptoms and therefore reduce dropouts from taking the IMP.

There is some evidence that PPIs may reduce the transport of metformin across cell membranes into hepatocytes.(Kim et al. 2014; Nies et al. 2011) The latter is mediated by OCTs including OCT1, which PPIs inhibit, reducing hepatic metformin levels.(Kim et al. 2014; Nies et al. 2011) OCT3 is found on the apical membrane of enterocytes and OCT2 on the basolateral membrane of the renal tubules.(Koepsell 1998) This potential drug interaction is important as nearly two thirds of PDAC patients were prescribed PPIs. Metformin uptake into the liver is mediated by the membrane transporter OCT1, and to a lesser extent by OCT3.(Graham et al. 2011)

PPIs were potent inhibitors of OCT-mediated metformin uptake into the liver in an in vitro study.(Nies et al. 2011) The half-maximal inhibitory concentration (IC_{50}) of omeprazole, pantoprazole, lansoprazole and rabeprazole were all in the micromolar range, indicative of potent OCT inhibition.(Nies et al. 2011) In most participants in our series there was no clear clinical reason documented for the PPI prescription, throughout the illness. Consideration of PPI use is important for a trial as it may lower metformin levels in the liver, so there is less inhibition of gluconeogenesis. In humans, whether the inhibition of metformin uptake into the liver by PPIs has a long term clinical effect on glycemic control is uncertain. A trial of 24 participants reported no statistical difference in glucose concentrations 180 minutes after ingestion, between those prescribed metformin, with or without a PPI.(Kim et al. 2014) Similarly, a double blind, randomized crossover, placebo-controlled trial of 20 healthy males receiving metformin, with placebo or lansoprazole 30mg, reported increased mean metformin maximum plasma concentrations in those co-administered a PPI over a 24 hour period, but there was no effect on maximum glucose levels.(Ding et al. 2014) Further pharmacodynamic studies are needed to confirm if the mechanism for how PPIs may reduce hepatic metformin uptake do affect long-term plasma glucose concentrations and hence insulin secretion. If there are drug interactions, an option is prescribing the H2 receptor antagonist, cimetidine, which does not impair hepatic metformin uptake and also beneficially reduces metformin renal excretion and raises plasma metformin concentrations.(Somogyi and Muirhead 1987)

This observational study had several strengths and limitations. Participant generalizability was assessed as many patients diagnosed with PDAC were studied over a two-year period. The demographics including: age at diagnosis, gender and survival time were as expected, as was the anatomical distribution of PDAC, with the

highest proportion of tumors located in the head of the pancreas. The measurement error for variables was low, as by using secondary care case notes, we accessed many other information sources including: primary care referral letters and results of multiple investigations. The limitations were participants were recruited from a single hospital site and the data were collected retrospectively. Information on symptoms was identified predominantly from outpatient clinic letters and their under-reporting is possible. However, the later would give a false under-estimate, rather than spurious over-estimates of our findings.

A future trial should consider the role of genetic polymorphisms, which may influence the pharmacokinetics and pharmacodynamics of metformin. The presence of the minor allele C of the ataxia-telangiectasia mutated gene located on chromosome 11 means a PDAC patient is more likely to respond to metformin, as this allele upregulates adenosine monophosphate-activated protein kinase (AMPK), which lowers blood glucose.(Zhou et al. 2011) Minor allele C is present in 44% of the population,(Zhou et al. 2011) and to the best of our knowledge, there are no case series, which have investigated its prevalence in PDAC.

In conclusion, the three main findings of our work to help justify and plan a future phase III trial of metformin in patients with inoperable PDAC without liver metastases are: i) one third of all patients with inoperable PDAC are eligible for a trial, ii) nearly two thirds of eligible participants had nausea and vomiting during follow-up, although most had no underlying identifiable cause. Appropriate management of these symptoms is important to reduce discontinuation of the study drug and iii) nearly two thirds of eligible participants are prescribed a PPI at diagnosis or during follow-up. Further pharmacodynamic studies need to clarify if the mechanisms on how PPIs may reduce hepatic metformin uptake lead to long-term effects on glucose

metabolism and insulin secretion. Investigating new safe, well tolerated oral chemotherapies in PDAC is vital to improve the current dismal prognosis of this aggressive malignancy.

References

Broadhurst PJ, Hart AR (2018) Metformin as an Adjunctive Therapy for Pancreatic Cancer: A Review of the Literature on Its Potential Therapeutic Use Digestive diseases and sciences 63:2840-2852 doi:10.1007/s10620-018-5233-y

Chatterjee S, Khunti K, Davies MJ (2017) Type 2 diabetes Lancet 389:2239-2251 doi:10.1016/s0140-6736(17)30058-2

Conroy T et al. (2016) Current standards and new innovative approaches for treatment of pancreatic cancer Eur J Cancer 57:10-22 doi:10.1016/j.ejca.2015.12.026

Conroy T et al. (2011) FOLFIRINOX versus Gemcitabine for Metastatic Pancreatic Cancer New England Journal of Medicine 364:1817-1825 doi:10.1056/NEJMoa1011923

Dello SAWG, Stoot JHMB, van Stiphout RSA, Bloemen JG, Wigmore SJ, Dejong CHC, van Dam RM (2011) Prospective volumetric assessment of the liver on a personal computer by nonradiologists prior to partial hepatectomy World journal of surgery 35:386-392 doi:10.1007/s00268-010-0877-6

Ding Y et al. (2014) The effect of lansoprazole, an OCT inhibitor, on metformin pharmacokinetics in healthy subjects European Journal of Clinical Pharmacology 70:141-146 doi:10.1007/s00228-013-1604-7

Ferlay J SI, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F. (2013.) GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. International Agency for Research on Cancer. http://globocan.iarc.fr. Accessed 08/09/2017

Gonzalez-Guindalini FD, Botelho MP, Harmath CB, Sandrasegaran K, Miller FH, Salem R, Yaghmai V (2013) Assessment of liver tumor response to therapy: role of quantitative imaging Radiographics : a review publication of the Radiological Society of North America, Inc 33:1781-1800 doi:10.1148/rg.336135511

Graham GG et al. (2011) Clinical pharmacokinetics of metformin Clinical pharmacokinetics 50:81-98 doi:10.2165/11534750-000000000-00000

Kim A et al. (2014) Effects of proton pump inhibitors on metformin pharmacokinetics and pharmacodynamics Drug metabolism and disposition: the biological fate of chemicals 42:1174-1179 doi:10.1124/dmd.113.055616

Koepsell H (1998) ORGANIC CATION TRANSPORTERS IN INTESTINE, KIDNEY, LIVER, AND BRAIN Annual Review of Physiology 60:243-266 doi:10.1146/annurev.physiol.60.1.243

Kordes S et al. Metformin in patients with advanced pancreatic cancer: a double-blind, randomised, placebo-controlled phase 2 trial The Lancet Oncology 16:839-847 doi:10.1016/S1470-2045(15)00027-3

Mantatzis M, Kakolyris S, Amarantidis K, Karayiannakis A, Prassopoulos P (2009) Treatment response classification of liver metastatic disease evaluated on imaging. Are RECIST unidimensional measurements accurate? European radiology 19:1809-1816 doi:10.1007/s00330-009-1327-4

Nies AT, Hofmann U, Resch C, Schaeffeler E, Rius M, Schwab M (2011) Proton pump inhibitors inhibit metformin uptake by organic cation transporters (OCTs) PloS one 6:e22163-e22163 doi:10.1371/journal.pone.0022163

Reni M et al. (2016) (Ir)relevance of Metformin Treatment in Patients with Metastatic Pancreatic Cancer: An Open-Label, Randomized Phase II Trial Clinical Cancer Research 22:1076-1085 doi:10.1158/1078-0432.ccr-15-1722

Rothe JH et al. (2013) Size determination and response assessment of liver metastases with computed tomography—Comparison of RECIST and volumetric algorithms European Journal of Radiology 82:1831-1839 doi:https://doi.org/10.1016/j.ejrad.2012.05.018

Sasaoka T, Rose DW, Jhun BH, Saltiel AR, Draznin B, Olefsky JM (1994) Evidence for a functional role of Shc proteins in mitogenic signaling induced by insulin, insulin-like growth factor-1, and epidermal growth factor J Biol Chem 269:13689-13694

Somogyi A, Muirhead M (1987) Pharmacokinetic Interactions of Cimetidine 1987 Clinical pharmacokinetics 12:321-366 doi:10.2165/00003088-198712050-00002

Suzuki C et al. (2008) Radiologic measurements of tumor response to treatment: practical approaches and limitations Radiographics : a review publication of the Radiological Society of North America, Inc 28:329-344 doi:10.1148/rg.282075068

Wang L-W, Li Z-S, Zou D-W, Jin Z-D, Gao J, Xu G-M (2008) Metformin induces apoptosis of pancreatic cancer cells World Journal of Gastroenterology 14:7192-7198 doi:10.3748/wjg.v14.i47.7192

Zhou G et al. (2016) Metformin Restrains Pancreatic Duodenal Homeobox-1 (PDX-1) Function by Inhibiting ERK Signaling in Pancreatic Ductal Adenocarcinoma Current molecular medicine 16:83-90

Zhou K et al. (2011) Common variants near ATM are associated with glycemic response to metformin in type 2 diabetes Nat Genet 43:117-120 doi:10.1038/ng.735