Expert Opinion

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Diabetes and fracture healing: the skeletal effects of diabetic drugs

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Introduction: Over 39,000 diabetic patients are surgically treated for trauma and orthopaedic injuries annually in the UK, yet the effects of diabetic medications on the skeletal system is an under researched and under acknowledged field.

Areas covered: This review covers all English language novel experimental data reports investigating the effects of the main classes of diabetic drugs on the skeletal system, specifically their effects on fracture healing, located through the literature search engines Medline and Web of Science.

Expert opinion: Post-surgical gylcaemic control is paramount in insulin-controlled type 1 diabetic patients. Data on pharmacological control compounds used in type 2 diabetes are limited. Reports to date indicate thiazolidinediones to exert anti-osteogenic effects, in contrast to the observed osteogenic effects of biguanides. Ongoing research is desirable to guide future clinical recommendations.

Keywords: biguanide, diabetes, fracture (+/- healing), insulin, metformin, osteogenesis, rosiglitazone, thiazolidinedione

1. Introduction

It was estimated in 2008 that 2,442,000 people in England have diabetes, equating to 4.77% of the population, a figure expected to reach 6.40% (3,605,000) by 2025 [1]. Trauma fractures continue to be a major burden on National Health Service resources, exacerbated by the continuing increase in multiple high risk populations, including the obese [2] and diabetic populations. Annually, ~ 39,000 diabetic patients are being surgically treated with trauma and orthopaedic injuries [3]. With these high numbers and resultant large financial implications, it is essential to be mindful of the effects of diabetes and its pharmacological control agents in the clinical setting, with an aim to decrease the current observed levels of complications and poor clinical outcomes [4]. Glucose has recently been demonstrated a very strong predictive indicator of mortality in the initial trauma setting [5]. This study will look at its impact at a later stage of the trauma and orthopaedic care pathway.

Diabetes mellitus has long been known to have an effect on the skeletal system. This effect having previously been coined 'diabetic osteopathy' by Bouillon [6] includes a number of phenotypic features including an increased fracture risk [7] and subsequent poor fracture healing and bone regeneration [8].

Fracture healing occurs in three distinct stages. First the inflammatory stage, within which a clot forms around the fracture site allowing an influx of inflammatory cells followed by a subsequent cytokine cascade recruiting the necessary osteoblasts and chondroblasts. Within the second, reparative, stage these osteoblasts and chondroblasts form a soft callus that eventually becomes hard callus over 6 – 12 weeks. Within the final, remodelling stage the callus begins to mature and remodel itself.

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Article highlights.

- The diabetic population represents a significant proportion of the trauma and orthopaedic caseload; however, the effects of diabetic medications on the skeletal system, specifically fracture healing, are not widely known or acknowledged.
- Fourteen articles are reviewed, located from the literature search engines Medline and Web of Science.
- From the limited data available, post-surgical close glycaemic control in insulin-dependent type 1 diabetics appears to be paramount to limit any effects on fracture healing.
- Recommendations regarding the effects of thiazolidinediones and biguanides are limited by the severe shortage of primary research; however, the apparent anti-osteogenic effects of thiazolidinediones and osteogenic properties of biguanides should be acknowledged while further research is conducted.

This box summarises key points contained in the article.

Research into the diabetic phenotype more specifically demonstrates the fracture callus to be slow to appear and mature [8] and fracture healing times to be prolonged by up to 87% [9]. More recent research has revealed increased chondrocyte and osteoblast apoptosis in the diabetic patient [10], the chondrocyte apoptosis being TNF-α mediated [11], along with increased osteoclast survival, leading to destruction of early callus tissue and impeding fracture healing.

Many diabetics are on medication for their condition preventing the aforementioned phenotype, however. In these patients, it is more poignant to consider the effects of the medications being used to treat diabetes, which may exert their own effects on the skeletal system, a possibility considerably less well researched. In this study, therefore, the evidence with regard to the effects of the main classes of diabetic drugs on the skeletal system is investigated with specific reference to their effects on fracture healing. Their more comprehensively investigated effects on initial fracture risk are not evaluated.

2. Materials and methods

The literature databases 'Medline' and 'Web of Science' were searched for all combinations of the words diabetes, fracture (+/- healing), osteogenesis, insulin, sulphonylureas, gliclazide, glibenclamide thiazolidinediones, rosiglitazone, pioglitazone, biguanides and metformin on the '18 April 2011.'

Inclusion criteria were articles in English language referring to the comparison of treated diabetic cohorts with untreated and non-diabetic cohorts, with respect to osteogenesis and fracture healing. Both *in vivo* experimental and clinical articles were included. Exclusion criteria were manuscripts not in English language, review articles and articles not demonstrating novel experimental data fulfilling the above criteria, that is, those not specifically investigating post-fracture healing.

Further articles, identified from the reference list of manuscripts, found to fulfil the inclusion criteria were also included in this review.

Such details were extracted and analysed as the type of the study (clinical or experimental), animal model of investigation, parameters of fracture healing assessed, the time points of assessment, type of pharmacological agent used as well as the findings and the conclusions derived out of the studies. In order to appropriately structure the review, results were divided according to pharmacological therapy and sub-divided as experimental or clinical.

3. Review of evidence

After strict application of the above criteria, out of 230 potentially eligible articles, 14 [7-10,12-21] were found to fulfil the specific search criteria detailed above (**Figure 1**). With regard to the effects of sulphonylureas on the skeletal system, no evidence fulfilling the above criteria was found, and hence the exclusion of this class of diabetic treatment from the study. The accumulated evidence regarding the other classes of medication is summarised in Tables I, II and III.

3.1 Insulin

Insulin normalises the metabolic failure observed in diabetes and thus as expected also normalises indices of mineralisation and callus bone content during fracture healing [22]. The gross observation of improved bone bridging with insulin-mediated diabetic control has been reported in both femoral [12] and calvarial osteotomy [13]. These findings have been repeated more recently by Kayal *et al.* [23] who demonstrated insulin to normalise post-fracture callus bone formation in diabetic mice.

The glycolysation products of collagen have been shown to enhance osteoclast-induced bone resorption [24] and also affect both enzymatic and non-enzymatic crosslinking in bone, impairing its mechanical properties [25]; the inverse of these findings is a reduction in 'advanced glycation end products,' achieved through insulin treatment, normalising resorption and mechanical properties.

More detailed bone histomorphometric parameters observed by Follak *et al.* [26] reiterated the above findings of insulin-mediated metabolic control significantly improving fracture healing. Fluorochrome-based parameters of mineralisation, apposition, formation and timing of mineralisation were all normalised in metabolically well-controlled subjects.

Recent research has focused on osteogenic genes in diabetes and the effects of diabetic treatments on their expression. Runt-related transcription factor 2 (RUNX2) and several RUNX2 target genes including MMP-9 are all downregulated in hyperglycaemic diabetic animals; however, insulin treatment significantly restores their expression [14].

The above research demonstrates how insulin therapy can rescue diabetic osteopathy at a genetic, molecular

A. <u>Medline</u>
1. Diabetes – 204063 rsults
2. Fracture (+/- healing) or osteogenesis – 83551 results
3. 1 and 2 – 860 results

4. 3 and 'insulin' -167 results (Articles meeting the inclusion criteria)
   - Beam *et al.* 2002
   - Follack *et al.* 2005
   - Follak *et al.* 2004
   - Fowkles *et al.* 2008
   - Kayal *et al.* 2009

5. Thiazolidinedione or rosiglitazone or pioglitazone – 5432 results
6. 3 and 5 – 43 results (Articles meeting the inclusion criteria)
   - Berberoglu *et al.* 2007

7. Biguanide or metformin – 5583 results
8. 3 and 7 – 18 results (Articles meeting the inclusion criteria)
   - Cortizo *et al.* 2006

9. Sulphonylurea or gliclazide or glibenclamide – 6449 results
10. 3 and 9 – 2 results
    - Neither fulfil criteria

B. <u>Web of science</u>
1. Searching method implemented as above but no new articles found meeting inclusion criteria

2. 'Cited by' tool used on all papers so far identified:
   - i. Gao *et al.* 2010 – cites Cortizo *et al.* 2006
   - ii. Kanazawa *et al.* 2008 – cites Cortizo *et al.* 2006
   - iii. Molinuevo *et al.* 2010 – cites Cortizo *et al.* 2006
   - iv. Berberoglu *et al.* 2010 – cites Berberoglu *et al.* 2007
   - v. Santana *et al.* 2003 – cited by Follak *et al.* 2004

3. 'Related records' tool used on all papers so far identified:
   - i. Grey *et al.* 2007 – related with Berberoglu *et al.* 2007

Figure 1. Search methods.

and tissue level; thus, post-fracture close metabolic control is essential in insulin-controlled diabetics. To date, no clinical research into the effects of interventions ensuring close glycaemic control has been undertaken.

3.2 Thiazolidinediones

Thiazolidinediones exert their effect through stimulation of the PPAR-γ receptor causing a decrease in peripheral insulin resistance and hepatic glucose output [15]. However, PPAR-γ is also expressed on bone marrow pluripotent mesenchymal stem cells, which can differentiate into adipocytes or osteoblasts, and stimulation may cause preferential differentiation into adipocytes over osteoblasts [16]. Thiazolidinediones have also been implicated in having an effect on fracture healing through their inhibitory effects on oestrogen and androgen biosynthesis [27].

Recent research has elucidated that thiazolidinediones suppress the bone anabolic signalling pathway as a result of PPAR-γ stimulation by decreasing the activity of Wnt, bone morphogenic protein (BMP) and IGF-I

pathways, while inducing production of RANKL, a cytokine supporting osteoclast development [17]. The accumulative result of the above is the decreased osteoblast number, decreased new vessel formation and large quantities of adipocytes observed at the healing site by Lecka-Czernik [18].

An *in vivo* rat model demonstrated the activation of PPAR-γ to inhibit osteoblastic differentiation and the expression of several anabolic mediators involved in bone formation [19]. The aforementioned study also observes *in vivo* decrease of tibial bone mineral density following treatment with rosiglitazone.

A significant decline in levels of bone formation markers osteocalcin, procollagen type I N-terminal propeptide and total alkaline phosphatase has been reported in subjects receiving rosiglitazone in two randomised controlled trials in humans [20,21]. It must be noted, however, that both these studies were conducted in post-menopausal women and thus cannot be extrapolated to the thiazolidinedione treated diabetic population as a whole.

A 2-year follow-up study conducted in the same population subset by Berberoglu *et al.* [28] demonstrated rosiglitazone treatment to adversely affect bone formation over a 2-year period and also to increase bone loss at the lumbar spine and trochanter.

As suggested by Lecka-Czernik [18], further clinical trials are necessary to assess the risk of skeletal complications in glitazone users of all demographics, but may in the future caution against thiazolidinedione use during a period of fracture healing.

The recent removal from licence of rosiglitazone further complicates the research in this area; however, it would be pertinent to assume pioglitazone to have similar skeletal effects until research is conducted that may or may not prove otherwise.

3.3 Biguanides

Metformin works through increasing insulin sensitivity, suppressing hepatic glucose production and enhancing peripheral glucose uptake. More importantly in the context of this review, it has been shown to have a direct osteogenic effect in a model of osteoblasts in culture through a dose-dependent increase in cell proliferation, type I collagen production and alkaline phosphatase activity. Markedly increased nodules of mineralisation were also noted at 3 weeks. In this study, metformin was shown to induce activation of phosphorylated extracellular signal-regulated kinase, as well as stimulating the expression of NOS; thus, these were hypothesised as the mediators of this osteogenic action [29].

A more recent study by Kanazawa *et al.* [30] confirmed the above findings while also noting a dose- and time-dependent increase in activation of the AMPK pathway and induction of eNOS and BMP-2 expression.

Molinuevo *et al.* [31] looked at both the *in vitro* effects of metformin, similar to the above studies, and *in vivo* effects in streptozotocin-induced diabetic rats. *In vitro* the above findings of features of increased osteoblastic differentiation were replicated while *in vivo* metformin administration was shown to enhance the expression of osteoblast-specific transcription factor Runx2/Cbfa1 and activate AMPK in a time-dependent manner.

A longer time-frame of metformin exposure *in vivo* was investigated by Gao *et al.* [32] who found the impaired bone density and quality in diabetic rats were significantly improved through treatment with metformin and this was possibly mediated through regulation of bone marrow progenitor cell development through induction of mechanisms regulating osteoblast markers Cbfa-1 and LRP5.

Metformin *in vitro* at clinically relevant doses has been shown to partially block the proadipogenic effect of rosiglitazone on bone marrow progenitor cells; however, it is not possible to extrapolate this finding to human patients [31]. This does open up an interesting and extremely clinically relevant avenue of future research, however, with the possibility of postoperative concomitant prescribing to be investigated.

4. Conclusions

There is substantial evidence indicating the significant effects of diabetic drugs on the skeletal system and fracture healing specifically. As yet, the majority of research is either in *in vitro* or *in vivo* animal models of diabetes with an extremely limited number of randomised controlled trials in humans. Further research is desirable regarding the effects of each of these drugs for all diabetic population subsets. This research should be conducted in large-population randomised controlled trials so as to maximise impact and have sufficient statistical power to be able to influence future clinical guidelines.

Research directly comparing the effects of biguanides and thiazolidinediones on fracture healing in humans should also be conducted so as to aid future prescribing guidelines in type 2 diabetes mellitus.

The possibility of post-fracture concomitant prescribing of metformin to a patient previously glycaemically controlled on rosiglitazone, to discover if this prevents the thiazolidinediones anti-osteogenic effects, as suggested by Molinuevo *et al.* [31], is a further area for investigation, within which the hypoglycaemic and side effect risks should be compared with the possible benefit to fracture healing in a risk–benefit paradigm.

5. Expert opinion

In the absence of substantial numbers of randomised controlled trials in humans, definitive statements regarding the use and prescription of diabetic drugs to patients in a fracture healing or bone repair process situation cannot be made. However, based on *in vitro* studies, animal data and the limited human trials conducted, certain recommendations can be made, and certain facts should be illustrated.

In insulin-controlled type 1 diabetic patients the importance of glycaemic control is paramount. Possible clinical actions relating to this include increased frequency of blood glucose monitoring or altering insulin regimens to maintain tighter control where possible. Future research into the efficacy of either of these methods is advised.

In pharmacologically controlled type 2 diabetes, less specific clinical recommendations are possible until further research that was suggested has been conducted. However, clinicians should be cognisant of the anti-osteogenic effects of glitazones and conversely osteogenic effects of metformin observed in studies to date when interpreting a clinical picture until further research-guided recommendations can be made.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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Supplementary material available online

Appendix 1: Tables I III. Appendix 2: Abbreviations.