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Defrosting insulin: a case report

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Summary: We report a case where a patient used microwave radiation to thaw his frozen insulin resulting in marked deterioration in his diabetic control. The consequences of this action were examined using high-performance liquid chromatography.

Introduction

Insulin is commonly stored under refrigeration, although it is well described that problems can arise when insulins are stored at temperatures below manufacturers' recommendations.[1,2] In contrast, other protein preparations stored within a hospital environment are often frozen; in this situation a rapid response for emergency situations may be achieved by controlled microwave thawing.[3] Concern has been expressed that the use of microwave radiation can lead to a reduction of functional plasma proteins due to inadvertent overheating.[4]

We report a case where a patient used microwave radiation to thaw his frozen insulin. We have examined the consequences of this action in terms of drug efficacy.

Case report

A 28 year old man with previously well-controlled insulin-dependent diabetes was seen in the clinic with a recent deterioration in his glycaemic control. Examination of his home capillary blood glucose chart showed that his usual good control had quite abruptly become erratic. Although the mean glucose value had risen only marginally from 8 to 10 mmol, wide fluctuations in values had produced subsequent loss of good control. This deterioration had continued over an 8 week period prior to his clinic attendance.

The patient volunteered no intercurrent illness, change in diet or circumstances to account for this unexpected worsening of glycaemic control. However, on direct questioning, the patient stated that, during an especially cold period of weather, his insulin (Actrapid MC/Novo, Lentard MC/Novo) had become frozen. He subsequently thawed both vials of frozen insulin on the 'defrost' mode of a domestic 600 W microwave oven and continued to use them. In retrospect he appreciated that his diabetes control had deteriorated since this event. A new prescription for replacement vials of Actrapid and Lentard insulins led to an immediate return to good control of his diabetes.

In order to explore the mechanism of the deterioration in glycaemic control, the same sequence of physical events was performed on vials of Actrapid and Lentard insulins. Vials of insulin were subjected to three separate physical environments. In Environment A, the insulin was frozen and then exposed to a microwave radiation thaw ('defrost' mode for 20 seconds). In B, the insulin was frozen and then left to defrost at the ambient room temperature (20°C), whilst in C, the control environment, insulin was stored at room temperature throughout. Macroscopic appearances were recorded and the *in vitro* potency of each vial of insulin was assayed by high-performance liquid chromatography (HPLC). This technique assesses the 'potency' of insulin by comparison of the elution pattern, from an absorbent column, of a recognized intact insulin standard against that of the insulin to be assayed. Elution speed is determined by molecular size and conformation which is only indirectly related to biological activity.

Macroscopically, all Actrapid insulin samples had a normal appearance. Both Lentard preparations exposed to freezing contained visible aggregates, in comparison to the Lentard which was stored in the control environment. The two experimental physical environments (A and B) did not affect the potency of either insulin preparation compared to the insulins in the control environment (C), when measured using HPLC. In contrast the Actrapid insulin exposed to both freezing and microwave radiation was found to contain a small

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quantity (0.3%) of a high molecular weight insulin component.

Discussion

It has been reported that the pharmacokinetics and the pharmacodynamics of lente and regular insulin mixtures are profoundly influenced by insulin vial storage temperature.[1] In our patient there may be several reasons why a deterioration in blood glucose control occurred. The Lentard which had been frozen contained aggregates and this may have influenced the *in vivo* timing characteristics of this long-acting insulin zinc suspension. In addition, the effect of the Lentard could have been altered because of variation in particle size after freezing, making it difficult to withdraw a uniform dose from the vial.

The action of insulin may additionally have been altered by protein denaturation, a recognized problem caused by local heating effects that can occur using microwaves.[3] This was assessed in our study *in vitro* using HPLC. Although there was no significant reduction in potency as assessed by this technique, there was evidence that a low level of decomposition of the Actrapid insulin did occur in the microwave-thawed sample (Environment A); this was not found in the Lentard insulin similarly exposed to microwaves. The fact that such little denaturation occurred could be attributed to the mode of microwave radiation used. The 'defrost' mode consists of brief pulses of heating followed by recovery periods, preventing excess local heating and hence denaturation.

We conclude that in our patient's case, most of the variation of glycaemic control resulted from changes in the physical characteristics of insulin which was secondary to freezing of the Lentard and probably not related to the microwave radiation. It is important to note that whilst HPLC is the accepted standard within the pharmaceutical industry for assessing insulin potency *in vitro*,[5] it does not necessaily reflect the bioavailability of insulin *in vivo*. Correct insulin storage is important for diabetic control and patient education should continue to emphasize this.

Acknowledgement

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