Cytochrome P450 (CYP) enzymes are important for drug metabolism. They chemically modify drugs to make them more soluble, which allows the drugs to be excreted from the body. Of the 30 CYP enzymes, 6 (CYP1A2, 2C9, 2C19, 2D6, 2E1, 3A4/5) are of critical interest because these enzymes are the key players in drug metabolism. Differences in genetics lead to individuals of different enzyme types. Metabolites of duloxetine were produced by incubating duloxetine with rat liver microsomes. Duloxetine is an antidepressant metabolized by CYP1A2 and CYP2D6 isotypes. In this experiment, Duloxetine was metabolized using rat microsomes, which are a variety of CYP enzymes.

Materials & Methods

Duloxetine can be metabolized in vitro when incubated with rat microsomes. Duloxetine metabolism in the presence of CYP inhibitors was manipulated by adding a general cytochrome P450 inhibitor, SKF 525A, and other isotype specific CYP inhibitors. Duloxetine was incubated with microsomes and NADPH-generating system from the procedure, as well as cleaned up the sample. The sample contained 50 µl of additional buffer. Experimental controls included a sample containing only duloxetine and buffer and a sample with only SKF 525A and buffer. Tubes were incubated for 2 hours. After incubation, 250 µl of methanol was added to the reaction. Duloxetine was centrifuged at top speed at 20°C for 20 minutes. The supernatant from each tube was extracted and placed in a new tube. The tubes were stored in the freezer until they were run on the HPLC-MS. HPLC separates the components of a mixture and uses absorbance to identify them. Components of the mixture can be identified and separated based on their mass to charge ratio by the mass spectrometer. A gradient of 5 mM ammonium acetate in water and 5 mM ammonium acetate in acetonitrile and water (95:5) was used. The gradient start at 95% ammonium acetate in water and decreased to 5%. The run time was 35 minutes for each sample. The sample contained 50 µl of incubation mixture diluted with 1 ml of ammonium acetate in water. The sample was run through the HPLC connected with the mass spectrometer and the type and percentage of each metabolite was determined using SW and TIC filtered data.

Results

Figure 1: Duloxetine metabolites can be identified and separated based on their mass to charge ratio by the mass spectrometer. A gradient of 5 mM ammonium acetate in water and 5 mM ammonium acetate in acetonitrile and water (95:5) was used. The gradient start at 95% ammonium acetate in water and decreased to 5%. The run time was 35 minutes for each sample. The sample contained 50 µl of incubation mixture diluted with 1 ml of ammonium acetate in water. The sample was run through the HPLC connected with the mass spectrometer and the type and percentage of each metabolite was determined using SW and TIC filtered data.

Figure 2: Duloxetine metabolites can be identified and separated based on their mass to charge ratio by the mass spectrometer. A gradient of 5 mM ammonium acetate in water and 5 mM ammonium acetate in acetonitrile and water (95:5) was used. The gradient start at 95% ammonium acetate in water and decreased to 5%. The run time was 35 minutes for each sample. The sample contained 50 µl of incubation mixture diluted with 1 ml of ammonium acetate in water. The sample was run through the HPLC connected with the mass spectrometer and the type and percentage of each metabolite was determined using SW and TIC filtered data.

Discussion

The peaks in the chromatogram were more spread out than expected, and the intensity of the peaks was relatively low. This may be due to duloxetine and its metabolites sticking to the C18 column, or the peak loss at some step in the procedure. Future experiments would aim to get single, more pronounced peaks for each metabolite in the HPLC and mass spectrum data. This could possibly be achieved by modifying the gradient parameters on the HPLC, using one single solvent instead of a gradient, or injecting the sample directly into the mass spectrometer instead of using the HPLC-MS combined.

By analyzing the total ion chromatogram, we find that duloxetine without the inhibitor showed 100% metabolism. Metabolism was reduced by 10% in the presence of CYP inhibitor, SKF 525A. In both cases metabolism was high, most likely because there was a high concentration of rat microsomes added to both samples. Previous studies, completed by Dani Scherluss, diluted the microsomes with phosphate buffer. For this experiment, 25 µl of undiluted rat microsomes were added to the incubation mixture, producing very high rates of metabolism. Another manipulation to the protocol was the removal of the NADPH-generating system. Previous research also showed the NADPH-generating system was not necessary to metabolize duloxetine. Removal of this system reduced the cost of the experiment, eliminated steps to create the NADPH-generating system from the procedure, as well as cleaned up the sample overall. An increase in CYP inhibition and metabolism production could be seen in future experiments if the concentration of SKF 525A was increased, rat microsome concentration was decreased, or isotype specific CYP inhibitors were used.

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